Malacothamnus

Volume 2

Keir Morse

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A Phylogenetic Assessment of Taxonomic Boundaries in the Genus *Malacothamnus* (Malvaceae)

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A Phylogenetic Assessment of Taxonomic Boundaries in the Genus *Malacothamnus* (Malvaceae)

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Author's Note

The three volumes of this *Malacothamnus* monograph mostly follow the three chapters of my PhD dissertation but with multiple revisions and additional peer review. The earlier dissertation version of this work should be viewed as an unfinished draft of what is presented here.

Three major goals in self-publishing my *Malacothamnus* research were to provide all the details future *Malacothamnus* researchers may need for their research, provide a large amount of color figures, and to make it all freely available to anyone who wanted it without spending thousands of dollars to do so. These goals are at odds with the way most academic publishing works at present and I could not find a journal that met the requirements necessary for these publications.

Regarding peer-review, I was faced with trying to satisfy multiple audiences that wanted different things out of these publications. Some people wanted all the details of the analyses and the evidence I used in making taxonomic decisions related to *Malacothamnus*. Some wanted a highly abridged form of this. Some just wanted the taxonomic treatment with lots of photos and some basic information about the genus. I can satisfy the first and last of those groups and I attempt to do so in this three-volume, open-access monograph of the genus. Those who want a highly abridged account can start with the abstracts and Volume 3, and then dig deeper into Volumes 1 and 2 if further interested.

Volume 1 is a morphological assessment of *Malacothamnus* to clarify how morphologically distinct previously described taxa and possible undescribed taxa are. These analyses resulted in specimen groupings with varying degrees of morphological and geographic cohesion, most of which align with previously described taxa.
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Volume 2 uses phylogenetic analyses to test the morphological groupings from Volume 1 as hypothesized lineages. The resulting evidence from both morphological and phylogenetic analyses are then used to make taxonomic decisions for the treatment in Volume 3. Areas where further research is needed are noted and three new species are described.
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Volume 3 is a new treatment of *Malacothamnus*, which includes preliminary conservation assessments of each taxon and natural history information related to the genus. It also summarizes some of the findings in Volumes 1 and 2 for those who do not wish to delve into the details in those volumes.
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Abstract

Past taxonomic treatments of the genus *Malacothamnus* (Malvaceae) are inconsistent with between 11 and 28 taxa recognized. Many taxa that are not recognized in recent treatments are narrowly endemic and of conservation concern, which makes resolving taxonomic questions in the genus a conservation priority. As a first step in resolving these questions, I used morphological analyses to assess specimens representing the full range of morphological variation in the genus throughout its entire geographic range, which is presented in Volume 1 of this monograph. These analyses resulted in 38 morphologically and geographically cohesive groups that have varying levels of distinctness.

In Volume 2 of this monograph, 36 of the 38 morphological groups are further assessed in a phylogenetic framework using a restriction site-associated DNA sequencing (RAD-seq) dataset. Samples were analyzed using maximum likelihood analysis, quartets-based species tree analysis, neighbor-net analysis, and multidimensional scaling. Resulting clades generally correspond with morphological groups and relationships between clades correspond as much to geographic patterns as morphological patterns. Sixteen of the 38 morphological groups formed well supported clades, with three additional well supported clades containing multiple morphological groups. Lower support and inconsistency between some clades and morphological groups indicates possible gene flow or incomplete lineage sorting between morphological groups in some regions and identifies focal areas for future research.

Taxonomic changes with a discussion of evidence for such changes are made in this volume. Based on the combination of morphologic, phylogenetic, and geographic evidence, I recognize 29 minimum rank taxa within 21 *Malacothamnus* species. Of these, 15 are currently listed as taxa of conservation concern, nine of which were not recognized in the recent Flora of North America treatment. Three new species are described: ***Malacothamnus astrotentaculatus***, ***Malacothamnus eastwoodiae***, and ***Malacothamnus discombobulatus***. Taxonomic changes include two new combinations and two changes of rank resulting in the following new names: ***Malacothamnus arcuatus* var. *elmeri***, ***Malacothamnus fremontii* var. *exfibulosus***, ***Malacothamnus jonesii* var. *gracilis***, and ***Malacothamnus jonesii* var. *niveus***. A new treatment of *Malacothamnus* following the resulting taxonomy of Volume 2 is presented in Volume 3.

1 INTRODUCTION

Malacothamnus Greene (subfamily Malvoideae, Malvaceae) is a genus of generally short-lived, fire-following shrubs found primarily in the California Floristic Province of North America (Bates 1963). The taxonomy of the genus has been controversial for many years due to conflicting treatments and the many taxa of conservation concern not recognized in some of these treatments (Kearney 1951; Skinner et al. 1995; Slotta 2012; Bates 2015; CNPS 2022). For those wishing to take a more conservation-minded approach and recognize the many *Malacothamnus* taxa of conservation concern, Kearney's (1951, 1955) treatment has been the standard to follow, which recognizes 28 taxa. For those with more of a taxonomic lumper mentality, the treatments of Bates (1993, 2015) offer the opposite extreme with only 11 taxa recognized. Slotta (2004, 2012) offers a somewhat intermediate interpretation with 17 taxa recognized. Figure 1 shows a comparison of these three treatments relative to all basionyms in what is currently recognized as *Malacothamnus*.

To resolve the question of which taxa should be recognized, both morphological (Bates 1963; Benesh and Elisens 1999; Slotta 2004; Morse and Chester 2019; Morse 2021, 2023) and molecular evidence (Swensen et al. 1995; Slotta 2004) have been employed. Additionally, Bates (1963) conducted chromosome counts, a crossing study, and a common garden experiment.

Bates (1963, 1968) classified three genera in the tribe Malveae as the *Malacothamnus* alliance (*Malacothamnus*, *Iliamna* Greene, and *Phymosia* Desvaux) based on chromosome numbers. He noted that both *Malacothamnus* and *Phymosia* were the only genera in Malveae to have a chromosome number of n = 17. All counts of *Iliamna* were n = 33, which is one less than 17 doubled. Bates suggested *Iliamna* is a polyploid derivative of a *Phymosia*-like ancestor.

Bates (1963) reported chromosomes counts of most of Kearney's (1951, 1955) 28 *Malacothamnus* taxa as well as putative wild hybrids and F_1 garden hybrids. All counts were n = 17, indicating an apparent lack of polyploidy within the genus. It is unclear exactly how many of Kearney's 28 taxa Bates did chromosome counts on (22–26) as the location of most of his vouchers is currently unknown. Bates (1963) only identified these samples to his more-broadly defined taxa but included the county where each was collected, which narrows down the possible Kearney-taxa each could be. The garden crosses were between 12 parental populations that Bates believed to represent distantly related taxa based on morphological divergence. These 12 populations included 10 of the 28 taxa recognized by Kearney and 4 of the 11 taxa recognized by Bates (2015). All but one of these crosses were successful (the failure only having a single pollination) and F_1 hybrids had both 90–100% "visibly good pollen" and a high seed set. Based on this and apparent zones of hybridization between geographically adjacent taxa, Bates hypothesized that genetic interchange is possible between all species in the genus.

Despite the lack of postzygotic reproductive barriers between at least those taxa assessed, prezygotic reproductive barriers between *Malacothamnus* taxa commonly include spatial and temporal isolation. While there are examples of sympatric species with full (*M. jonesii* and *M. davidsonii*) or partial (*M. davidsonii* and *M. fasciculatus* var. *laxiflorus*) isolation based on bloom time, the primary temporal reproductive barrier may be related to the fire-following nature of the genus. *Malacothamnus* primarily germinate after fire and, in many

populations, all or most plants are dead and reduced to just a soil seed bank within 3–10 years post-fire (Morse, unpubl. data). Mean fire return intervals of pre-Euro-American settlement fire regimes in habitats supporting *Malacothamnus* ranged from ~10–150 years with a maximum of ~250 years (Van de Water and Safford 2011). Thus, smaller fires, possibly limited to a geographic area by physical and/or climatic variables, may create short-lived "islands" of *Malacothamnus* plants that would have no or little gene flow with plants from nearby areas that have not burned recently. This may allow for genetic drift and adaptation to the local environment. When a larger fire occurs that crosses geographic boundaries that smaller fires perhaps rarely cross, a large bloom of *Malacothamnus* could allow for short-term gene flow between areas where there is rarely gene flow. The combination of both small- and large-scale fires could allow for both speciation, in the case of the smaller fires, and a maintenance of hybrid zones between taxa, in the case of larger fires.

The only genus-wide phylogenetic assessment of *Malacothamnus* used the nuclear ITS region with both parsimony and maximum likelihood analyses and was unable to resolve relationships within the genus (Slotta 2004). Slotta additionally used parsimony analysis to examine the relationships of the three genera in the *Malacothamnus* Alliance with nine other Malveae genera using sequences from the nuclear ITS region and the plastid *rpL*16 intron, *trn*L intron, and the *trn*L-*trn*F intergenic spacer. In all analyses, *Malacothamnus* was resolved as monophyletic. ITS, which provided the phylogeny with the highest statistical support, resolved *Phymosia* as monophyletic and *Iliamna* as two separate clades. This phylogeny supports *Phymosia* as sister to *Malacothamnus* and the majority of *Iliamna* as sister to the pair of *Malacothamnus* and *Phymosia*. A larger nrITS study of Malveae using both parsimony and Bayesian analyses included 68 genera, but only a single sample each of *Malacothamnus* and *Phymosia*, and resolved a polytomy of *Malacothamnus*, *Phymosia*, and part of *Iliamna* in both types of analysis (Tate et al. 2005).

Additional molecular studies have been done in relation to two federally listed taxa, *Malacothamnus clementinus* and *M. fasciculatus* var. *nesioticus*. The *M. clementinus* studies assessed genetic diversity within and between populations of the species (Helenurm 1997, 1999). The study of *M. fasciculatus* var. *nesioticus* included all five varieties of *M. fasciculatus* (Swensen et al. 1995). This study used isozyme, randomly amplified polymorphic DNA, and ribosomal DNA analyses to evaluate the genetic distinctness of *M. fasciculatus* var. *nesioticus* relative to the other *M. fasciculatus* varieties. The authors note that, while the monophylly of each variety has not been established, their evaluation supports the morphologically based varietal distinctions. They point to unique RAPD allele combinations and isozyme alleles in *M. fasciculatus* var. *nesioticus* as evidence that it is genetically distinct from other conspecific varieties.

In Volume 1 of this monograph (Morse 2023), I defined 38 morphological groups based on both morphological and geographic cohesiveness. These morphological groups mostly conform to the 28 taxa recognized by Kearney (Fig. 1) plus 10 additional groups (Table 1). These 38 groups range from well-defined species that are both geographically isolated and morphologically distinct to intergrading forms needing further evidence to base taxonomic decisions upon. With a few exceptions of sympatry, most of these morphological groups are

allopatric and often have limited zones of contact where morphologically intermediate plants occur.

Departures in these morphological groupings from Kearney (1951, 1955) include a species described after Kearney's treatment, redefined taxon boundaries, geographically disjunct groupings of certain taxa, morphologically distinct groupings hypothesized as undescribed taxa, and possible intermediate groupings. The morphological characters distinguishing *Malacothamnus densiflorus* var. *viscidus* were redefined in Volume 1. *Malacothamnus foliosus* was split into an inland and coastal group of which the coastal group is what is typically thought of as *M. paniculatus*, but it isn't clear whether the type specimen of *M. paniculatus* better fits in the inland or coastal group. *Malacothamnus davidsonii*, *M densiflorus* s.s., and *M. fremontii* s.s. are each split into two groups based primarily on geographic disjunction. "Shasta" and "Vandenberg" plants are clearly both morphologically and geographically distinct from similar groups. *Malacothamnus enigmaticus* was described in 2019 and similar plants from Baja California are called "Baja East". "Santa Rosa Mountains" was a small group of specimens from the Santa Rosa Mountains of California that may be intermediate between *M. enigmaticus*, *M. fasciculatus* var. *laxiflorus* and/or *M. densiflorus* var. *densiflorus*. Additional intermediate groups include *M. fasciculatus*/*foliosus* intermediates and "Baja East"/*fasciculatus* intermediates.

In this volume, I test 36 of the 38 morphological groupings from Volume 1 as hypothesized lineages within a phylogenetic framework using a restriction site-associated DNA sequencing (RAD-seq) dataset. This dataset is analyzed using maximum likelihood (ML) analysis, quartets-based species tree analysis, neighbor-net analysis, and multidimensional scaling (MDS). The totality of evidence from both the morphological study of Volume 1 and this phylogenetic study is then used to assign names and ranks to the taxa/lineages that will be recognized in the new treatment of the genus (Volume 3) and to identify future research needs related to the genus.

Basionym with year published
Kearney (1951, 1955)
Slotta (2004, 2012)
Bates (1993, 2015)
Malvastrum abbottii Eastw. (1936)
M. abbottii (Eastw.) Kearney
M. abbottii
M. abbottii
Malvastrum aboriginum B.L.Rob. (1897)
M. aboriginum (B.L.Rob.) Greene
M. aboriginum
M. aboriginum
Malvastrum clementinum Munz & I.M.Johnst (1924)
M. clementinus (Munz & I.M.Johnst.) Kearney
M. clementinus
M. clementinus
Malvastrum davidsonii B.L.Rob. (1897)
M. davidsonii (B.L.Rob.) Greene
M. davidsonii
M. davidsonii
Malvastrum densiflorum S.Watson (1882)
M. densiflorus (S.Watson) Greene var. densiflorus
M. densiflorus
M. densiflorus
Malvastrum viscidum Abrams (1907)
M. densiflorus var. viscidus (Abrams) Kearney
Malva fasciculata Nutt. ex Torr. & A.Gray (1838)
M. fasciculatus (Nutt. ex Torr. & A.Gray) Greene var. fasciculatus
M. fasciculatus var. fasciculatus
M. fasciculatus
Malvastrum thurberi A.Gray (1854)
Malvastrum splendidum Kellogg (1855) and Malvastrum thurberi var. laxiflorum A.Gray (1886)
M. fasciculatus var. laxiflorus (A.Gray) Kearney
Malvastrum parishii Eastw. (1936)
M. parishii (Eastw.) Kearney
Malvastrum nesioticum B.L.Rob. (1897)
M. fasciculatus var. nesioticus (B.L.Rob.) Kearney
M. fasciculatus var. nesioticus
Malacothamnus nuttallii Abrams (1910)
M. fasciculatus var. nuttallii (Abrams) Kearney
M. fasciculatus var. nuttallii
Malvastrum catalinense Eastw. (1936)
M. fasciculatus var. catalinensis (Eastw.) Kearney
M. fasciculatus var. catalinensis
Malveopsis arcuata Greene (1894)
M. arcuatus (Greene) Greene
?
Malvastrum hallii Eastw. (1936)
M. hallii (Eastw.) Kearney
M. hallii
Sphaeralcea fasciculata var. elmeri Jeps. (1936)
Malvastrum mendocinense Eastw. (1939)
M. mendocinensis (Eastw.) Kearney
Malvastrum foliosum S.Watson (1885)
M. foliosus (S.Watson) Kearney
?
M. foliosus
Malvastrum marrubioides var. paniculatum A.Gray (1886)
M. paniculatus (A.Gray) Kearney
M. paniculatus
Malvastrum fremontii Torr. ex. A.Gray (1849)
M. fremontii (Torr. ex A.Gray) Greene
M. fremontii
M. fremontii
Malvastrum howellii Eastw. (1936)
M. howellii (Eastw.) Kearney
Malvastrum howellii var. cordatum Eastw. (1936)
Malvastrum fremontii var. cercophorum B.L.Rob. (1897)
Malvastrum helleri Eastw. (1936)
M. helleri (Eastw.) Kearney
Sphaeralcea fremontii var. exfibulosa Jeps. (1936)
Malvastrum orbiculatum Greene (1891)
M. orbiculatus (Greene) Greene
M. orbiculatus
Malvastrum jonesii Munz (1925)
M. jonesii (Munz) Kearney
M. jonesii
M. jonesii
Malvastrum dudleyi Eastw. (1936)
Malvastrum gracile Eastw. (1936)
M. gracilis (Eastw.) Kearney
Malvastrum niveum Eastw. (1936)
M. niveus (Eastw.) Kearney
Malvastrum marrubioides Durand & Hilg. (1855)
M. marrubioides (Durand & Hilg.) Greene
M. marrubioides
M. marrubioides
Malvastrum gabrielense Munz & I.M.Johnst. (1925)
Malvastrum palmeri S.Watson (1877)
M. palmeri (S.Watson) Greene var. palmeri
M. palmeri var. palmeri
M. palmeri
Malacothamnus palmeri var. lucianus Kearney (1955)
M. palmeri var. lucianus
Malvastrum involucratum B.L.Rob. (1897)
M. palmeri var. involucratus (B.L.Rob.) Kearney
M. palmeri var. involucratus
Malacothamnus enigmaticus K.Morse & T.Chester (2019)
(published after all treatments)

Figure 1. Taxonomic flowchart showing all basionyms currently included within the genus *Malacothamnus* and how each was treated by Kearney, Slotta, and Bates. *Malacothamnus* is abbreviated as *M.* for these treatments. *Malvastrum splendidum* and *Malacothamnus fasciculatus* var. *laxiflorus* are homotypic. Bates's species on the right represent the broadest published circumscriptions of *Malacothamnus* taxa by any author and the groupings where confusion lies between included taxa. Question marks denote two taxa where it is unclear how Slotta intended to treat them. Bates's unpublished 1963 treatment recognized all species from his 1993 and 2015 treatments as subspecies of *M. fasciculatus*.

Table 1. *Malacothamnus* morphological groups from Volume 1 (Morse 2023) and taxa they are assigned to in the new treatment of the genus (Volume 3) based on the combined evidence of Volumes 1 and 2. Taxa in bold include new species and taxonomic changes published in this volume. * denotes morphological groups needing further research. ** denotes two morphological groups not included in phylogenetic analyses, which also require further research.

Morphological Groups from Volume 1	Taxon Assignment for Volume 3 Treatment
M. abbottii (Eastw.) Kearney	*M. abbottii*
M. aboriginum (B.L.Rob.) Greene	*M. aboriginum*
M. arcuatus (Greene) Greene	*M. arcuatus* var. *arcuatus*
M. clementinus (Munz & I.M.Johnst.) Kearney	*M. clementinus*
M. davidsonii (B.L.Rob.) Greene - southern populations (type locality)	*M. davidsonii*
M. davidsonii - northern populations	***M. discombobulatus* K.Morse**
M. densiflorus (S.Watson) Greene var. *densiflorus* - San Jacinto Mountains (type locality)	*M. densiflorus* var. *densiflorus*
M. densiflorus var. *densiflorus* - remaining locations	*M. densiflorus* var. *densiflorus*
M. densiflorus var. *viscidus* (Abrams) Kearney	*M. densiflorus* var. *viscidus*
M. enigmaticus K.Morse & T.Chester	*M. enigmaticus*
M. fasciculatus (Nutt. ex Torr. & A.Gray) Greene var. *fasciculatus**	*M. fasciculatus* var. *fasciculatus*
M. fasciculatus var. *catalinensis* (Eastw.) Kearney	*M. fasciculatus* var. *catalinensis*
M. fasciculatus var. *laxiflorus* (A.Gray) Kearney*	*M. fasciculatus* var. *laxiflorus*
M. fasciculatus var. *nesioticus* (B.L.Rob.) Kearney	*M. fasciculatus* var. *nesioticus*
M. fasciculatus var. *nuttallii* (Abrams) Kearney	*M. nuttallii* Abrams
M. fasciculatus/*foliosus* intermediates*	Probably *M. fasciculatus* var. *fasciculatus* and *M. fasciculatus*/*foliosus* intermediates
M. foliosus (S.Watson) Kearney - inland form (type locality)*	*M. foliosus*
M. foliosus - coastal form*	*M. foliosus*
M. fremontii (Torr. ex A.Gray) Greene - southern populations (likely type locality)*	*M. fremontii* var. *fremontii*
M. fremontii (Torr. ex A.Gray) Greene - northern populations*	*M. fremontii* var. *fremontii*
M. gracilis (Eastw.) Kearney	***M. jonesii* var. *gracilis* (Eastw.) K.Morse**
M. hallii (Eastw.) Kearney	***M. arcuatus var. elmeri* (Jeps.) K.Morse**
M. helleri (Eastw.) Kearney	***M. fremontii* var. *exfibulosus* (Jeps.) K.Morse**
M. howellii (Eastw.) Kearney*	*M. fremontii* var. *fremontii*
M. involucratus (B.L.Rob.) K.Morse	*M. involucratus*
M. jonesii (Munz) Kearney	*M. jonesii* var. *jonesii*
M. lucianus (Kearney) K.Morse	*M. lucianus*
M. marrubioides (Durand & Hilg.) Greene	*M. marrubioides*
M. mendocinensis (Eastw.) Kearney	*M. mendocinensis*
M. niveus (Eastw.) Kearney	***M. jonesii* var. *niveus* (Eastw.) K.Morse**
M. orbiculatus (Greene) Greene	*M. orbiculatus*
M. palmeri (S.Watson) Greene	*M. palmeri*
M. parishii (Eastw.) Kearney**	*M. fasciculatus* var. *laxiflorus*
"Baja East"*	*M. enigmaticus*
"Baja East"/fasciculatus intermediates*	Probably intermediates of Baja California species
"Santa Rosa Mountains"**	Probably intermediates of *M. fasciculatus* var. *laxiflorus*, *M. enigmaticus*, and/or *M. densiflorus* var. *densiflorus*
“Shasta”	***M. astrotentaculatus* K.Morse**
“Vandenberg”	***M. eastwoodiae* K.Morse**

2 MATERIALS AND METHODS

2.1 Sampling

A total of 350 individuals of *Malacothamnus* were sampled for sequencing representing 36 of the 38 morphological groups from Volume 1 (Morse 2023) and possible hybrids (Appendix 1, Table 1). The two morphological groups not represented are *M. parishii* and a morphological group called "Santa Rosa Mountains" in Volume 1. *Malacothamnus parishii* is presumed extinct and only known from the type specimens collected in 1895 and possibly a second nearby collection from 1909. Sampling of these specimens is left to a future researcher using a sequencing strategy more suited to highly fragmented DNA. "Santa Rosa Mountains" includes a few specimens collected from the 1930s to 1960s and are possible intermediates between *M. fasciculatus* var. *laxiflorus*, *M. enigmaticus*, and/or *M. densiflorus* var. *densiflorus*. These were mistakenly overlooked when sampling specimens. A possible hybrid specimen from the Santa Rosa Mountains is included in this study but this specimen does not match the morphology of the Santa Rosa Mountains specimens measured in Volume 1. Ten outgroup taxa in Malveae and one in Hibisceae are represented including four from the *Malacothamnus* alliance: *Iliamna bakeri* (Jeps.) Wiggins, *I. latibracteata* Wiggins, *I. rivularis* (Douglas) Greene, and *Phymosia umbellata* (Cav.) Kearney. Additional outgroups include *Abutilon parvulum* A.Gray, *Eremalche rotundifolia* (A.Gray) Greene, *Hibiscus denudatus* Benth., *Malva parviflora* L., *Sida abutilifolia* Mill., *Sidalcea malviflora* (DC.) A.Gray, and *Sphaeralcea ambigua* A.Gray. Genomic DNA was extracted from the leaves of either silica-dried specimens collected in the field from 2017 to 2021 or from air-dried herbarium specimens collected from 1890 to 2021 (Appendix 1).

2.2 DNA Extraction, Library Preparation, and Sequencing

DNA was extracted from dried leaf material using a modified CTAB protocol (Doyle and Doyle 1987) using pectinase to reduce contaminating polysaccharides (Appendix 2). Four single-digest RAD-seq libraries were prepared using the restriction enzyme *Pst-1.* One library of 95 samples was prepared and sequenced at Floragenex on an Illumina HiSeq 2500 (San Diego, CA) using a single-end, mid-output, 100 bp kit. Library preparation of the remaining three libraries was conducted in-house at California Botanic Garden, Claremont, CA (Appendix 3). These three libraries were sequenced at the University of California, Riverside (UCR) Institute for Integrative Genome Biology Genomics Core Facility on an Illumina NexSeq 500 v2.5 (San Diego, CA) using a single-end, mid-output, 150 bp kit. Eight samples with few reads from their first sequencing were sequenced a second time in the final library. The reads of samples included in these four libraries were combined to produce a single alignment per sample.

2.3 Alignment Assembly and Quality Control

All four libraries were demultiplexed and assembled de novo with Ipyrad v. 0.9.17 (Eaton and Overcast 2020) using default settings except for trimming and cluster threshold. Sequences were trimmed to 80 bp, which is the approximate length of the 100 bp reads after removal of adapters and barcodes. Effects of the cluster threshold setting in iPyrad were explored by comparing a set of 25 samples run at cluster thresholds from 80 to 95. Parsimony informative sites peaked at 86 and variable sites peaked at 90. Exploratory ML trees run in IQ-TREE v. 1.6.12 (Nguyen et al. 2015) with various numbers of samples and cluster thresholds of 86, 88, and 90 all yielded similar trees with equally logical divergences. Most later analyses were run with the median cluster threshold of 88 with some comparisons to 86 and 90.

As one library was sequenced to 100 bp (Floragenex) and three were sequenced to 150 bp (UCR), an untrimmed ML tree of just the three 150 bp libraries (Fig. S1) was compared to trees of all four libraries trimmed to 80 bp to assess if the base pairs trimmed from the 150 bp libraries indicated different relationships than the full trimmed dataset. There was little difference in relationships produced from each dataset, thus all analyses use all libraries trimmed to 80 bp to keep the full dataset consistent between all libraries in analyses.

Data exploration found some samples with both relatively few sequencing reads and recovered loci to be inconsistent in placement in ML trees or to have highly questionable placement in clades compared to morphologically and geographically similar samples. To reduce problems associated with samples that had very low numbers of loci recovered, samples with less than 1000 recovered loci in an iPyrad alignment of all samples using a cluster threshold of 88 were excluded from all alignments used in post-exploratory analyses, including those of the “full” dataset. Outgroups generally had fewer loci recovered and were not removed despite two having fewer than 1000 loci (*Sidalcea malviflora* and *Iliamna latibracteata*).

Three main alignments were employed in this study. One contained all samples, which I refer to as the “full dataset with hybrids”. A second contained all samples classified to the morphological groups of Volume 1 (Morse 2023) excluding putative hybrids between these groups, which I refer to as the “full dataset without hybrids”. A third I refer to as the “reduced dataset”, which included three representatives, when available, for each of the morphological groups from Volume 1. These representatives were chosen based on having relatively low amounts of missing data compared to the full dataset while trying to maintain a good geographic sampling for each group. The reduced dataset includes seven, rather than three, samples of *Malacothamnus fasciculatus* var. *laxiflorus* which is split into eastern and western regions that early exploratory analyses indicated may not be closely related. Additional smaller alignments explored were focused on specific taxa.

Beyond the two included morphological groupings of Morse (2023) that possibly include hybrids (fasciculatus/foliosus intermediates and “Baja East”/fasciculatus intermediates), 35

additional samples were hypothesized to be of hybrid origin and excluded from the full dataset without hybrids. Most of these possible hybrids were morphologically and geographically intermediate plants between two or more groups. These include intermediates between the following pairs of groups: "Vandenberg" with *M. fasciculatus* var. *nuttallii*, *M. hallii* with *M. howellii*, *M. davidsonii* (south) with *M. fasciculatus* var. *laxiflorus, M. niveus* with two taxa (*M. jonesii* and *M. gracilis*), *M. densiflorus* var. *densiflorus* with three taxa (*M. enigmaticus*, *M. fasciculatus* var. *laxiflorus*, and *M. fasciculatus* var. *fasciculatus*), and *M. orbiculatus* with four taxa (*M. fasciculatus* var. *laxiflorus*, *M. fasciculatus* var. *nuttallii*, *M. fremontii*, and *M. marrubioides*). Possibly greater than two taxa are involved in some intermediates from the Santa Clarita region of California where the ranges of *M. marrubioides*, *M. orbiculatus*, and *M. fasciculatus* var. *laxiflorus* meet.

Additional likely hybrids from cultivated settings are included. Only two likely hybrids were found at California Botanic Garden, one of unknown parentage (M385) and one that came up under a *Malacothamnus aboriginum* (M390) before the parent plant (RSALC21614) died. Two of three samples from the University of California Botanic Garden at Berkeley (UCBG_65_1187 and UCBG_85_0039) fell into unexpected clades in the preliminary ML assessment of all samples, which indicates they may also be of hybrid origin. All three of these Berkeley specimens were treated as possible hybrids. A plant resembling *M. marrubioides* (M361) from a restoration planting of *M. abbottii* (misidentified as *M. jonesii*) grown by a commercial nursery selling multiple *Malacothamnus* taxa may possibly be of hybrid origin. Finally, plants were found growing in a disturbed area next to a building in Santa Cruz, CA that did not fit the morphology of any known species (M173). Plantings around this building were all California native plants, suggesting this could possibly be a cultivated hybrid.

Putative and likely hybrids were included in some analyses for two reasons. First, as the putative parents of many of these are known with little or no reason for doubt, this allowed me to use those as controls to test how they come out in analyses and whether these analyses are informative regarding which taxa may be the parental taxa of putative hybrids. Second, if the presumed parentage of these controls is clear in analyses and matches what is known, then the analyses may be trustworthy regarding identifying possible parental taxa in putative hybrids where the parentage is unknown such as garden hybrids and where more than two taxa are geographically adjacent.

2.4 Analyses

ML analyses were run on the various SNP alignments mentioned above using IQ-TREE with default settings and 1000 ultrafast bootstraps. ModelFinder (Kalyaanamoorthy et al. 2017), which is included within IQ-TREE, was used for substitution model selection. In the full dataset without hybrids, GTR+F+G4 was the best fit model under the Akaike Information Criterion, Corrected Akaike Information Criterion, and Bayesian Information Criterion, and was

thus used as the substitution model for all ML analyses. Clades with bootstrap values of ≥ 95% I consider well-supported and those with values of 70-94% I consider moderately supported. I use these two categories of bootstrap support (BS) values in the phylogenies to ease interpretation of the phylogeny. Bootstrap conflict between trees was examined in SplitsTree4 (Huson and Bryant 2006).

Quartets-based species tree analysis of SNP alignments was done using the Tetrad tool within Ipyrad using default settings and 1000 bootstraps.

A tanglegram comparing the ML and Tetrad trees of the reduced dataset was prepared in R using the *dendextend* v. 1.15.2 package (Galili 2015).

Neighbor-net analysis and MDS using uncorrected P-distances from unlinked SNP alignments was done to assess smaller groupings of samples outside of the limitations of a bifurcating tree. Neighbor-net analysis was performed using SplitsTree4. MDS was conducted in R v. 4.1.2 (R Core Team 2021) using the function *cmdscale* from distances generated in SplitsTree4.

ML and species tree analyses were run on the UCR High-performance Computing Cluster (HPCC). Neighbor-net analysis and MDS were run on a personal computer rather than a HPCC, which limited the number of samples that could be included and the number of bootstraps that could be run in neighbor-net analyses as visualization of the bootstrap results was computationally prohibitive at higher numbers. Bootstraps for neighbor-net analyses ranged from 50 to 1000 depending on the number of samples included.

A pruned phylogeny having only one tip for each morphological group, with the exception of two for the polyphyletic *Malacothamnus fasciculatus* var. *laxiflorus*, was created as a simplified representation of possible phylogenetic relationships between groups to compare geographic patterns and morphological characters between clades. This phylogeny is essentially the ML analysis of the reduced dataset with collapsed clades and some clade placement decisions based on patterns in multiple trees. As there is conflict between trees and not all branches are well supported, this pruned phylogeny should be considered representational of general patterns but not necessarily 100% accurate. This phylogeny is plotted with a map showing the collection locations of all wild-collected *Malacothamnus* samples included in the full dataset without hybrids. Sample points are color-coded to match the phylogeny tips and nodes are rotated to best illustrate the hypothesis that geographically adjacent taxa and clades may be more closely related to each other than those that are not geographically adjacent.

Morphological character states from Volume 1 (Morse 2023) were plotted against the pruned phylogeny tips representing morphological groupings to visualize which character states may correspond with phylogenetic relationships and to assess if any character states may be apomorphies of particular clades. Ancestral state reconstruction was not attempted. As many morphological groups had a high range of variability in character states (often with outliers) and

there was a high degree of overlap between characters in morphological analyses, these characters are simplified to better display the general patterns between each group. Quantitative characters use the mean values for each morphological group, which are divided into three categories using natural breaks when possible. Qualitative characters are mostly divided into three categories. Many already had just three categories. Inflorescence type, with six categories, was reduced to three. Characters assessing presence/absence were kept at two categories. Rather than using a mean value for qualitative characters, all character values represented in > 25% of specimens measured for a particular group are shown. This accommodates near and actual 50/50 splits between character states, removes outliers, and plots ≥ 75% of the variation observed. No characters with three categories had more than two categories accounting for ≥ 25% of specimens measured in any particular morphological group.

Phylogenies not noted above as being plotted in R or SplitsTree were plotted in FigTree v1.4.4 (Rambaut 2018). Maps were generated in QGIS 3.10.7 (QGIS Development Team 2020). Adobe Acrobat and Adobe Photoshop were used to edit the layout and enhance clarity of raw figures exported from FigTree, SplitsTree, R, and QGIS.

2.5 Taxonomic decisions

All taxonomic decisions for the *Malacothamnus* treatment in Volume 3 are made here based on the full evidence available. I followed the unified species concept and its companion subspecies concept in recognizing species as separately evolving metapopulation lineages and subspecies as incompletely separated lineages within a more inclusive lineage (de Queiroz 2007, 2020). De Queiroz (2020) argues that it is equally justifiable to call two incompletely separated lineages as subspecies (or varieties) of a single species where the infraspecific rank indicates the incompleteness of the separation, or to recognize them as separate species provided that there is some form of annotation noting the incomplete separation. Due to the inconsistent application of the ranks of variety and subspecies in botanical nomenclature (Hamilton and Reichard 1992), I consider these ranks to be generally equivalent in practice (both being incompletely separated lineages of a species) and follow most previous *Malacothamnus* treatments in using varieties. Some taxonomic decisions needed to be made when there was conflicting evidence or more evidence is still needed. In these cases, I use taxonomic ranks that will hopefully maximize practical application of names in field work and in databases while minimizing taxonomic upheaval until the more ambiguous evolutionary relationships between taxa can be resolved.

3 SEQUENCING AND ALIGNMENT RESULTS

Samples had an average of 1,052,739 reads that passed iPyrad filters (Table 2). The number of loci and amount of missing data varied by alignment (Table 3). Alignments including relatively few samples had a much higher average of recovered loci and a much lower amount of missing SNP sites. For example, the reduced dataset has ~2.5x as many recovered loci for the average sample and 12% less missing data than the full dataset without hybrids. This is mostly due to the inclusion of samples with a higher number of reads and exclusion of samples with a lower number of reads. While the high amount of missing data in Table 3 (77-90%) may raise red flags to those unfamiliar with RAD-seq, Tripp et al. (2017) showed increased BS in ML analysis of alignments that allow for more missing data and fully resolved the phylogeny of a genus using a RAD-seq dataset with 90% missing data.

Table 2. Data processing summary statistics from iPyrad for all samples using a clustering threshold of 88.

Stat	Raw reads	Reads passed filter	Total clusters	Clusters hi-depth	Hetero est	Error est	Reads consens	Loci in assembly
Max	5,413,245	5,403,949	204,106	127,618	0.0424	0.0144	104,098	26,612
Mean	1,053,542	1,052,739	107,719	51,029	0.0264	0.0049	40,902	6,795
Min	24,331	24,331	1,769	1,094	0.0011	0.0015	1,074	145
SD	765,638	764,409	38,270	24,868	0.0088	0.0028	20,817	4,893

Table 3. Comparison of loci numbers and missingness of data in SNP alignments.

			Loci recovered per sample (excluding outgroups)			
Alignment	# of samples included	Total retained loci after filters	Min	Mean	Max	SNP missing sites
all samples	361	98,462	145	6,932	26,612	90.5%
full dataset with hybrids	351	98,389	1,007	7,133	26,648	90.2%
full dataset without hybrids	316	97,606	1,153	7,953	28,989	89.3%
reduced data set	120	101,122	4,148	20,254	46,780	77.4%
M. fasciculatus varieties	101	102,699	3,443	21,750	54,749	77.5%

4 FULL GENUS ANALYSES

4.1 Maximum Likelihood Analyses

ML analysis of the full dataset without hybrids (Figs. 2-5) and the reduced dataset (Fig. 6) were mostly consistent. Monophyletic morphological groups with 100% BS in the tree from the reduced dataset and ≥ 95% BS in the tree from the full dataset without hybrids include: *Malacothamnnus abbottii*, *M. aboriginum*, *M. clementinus*, *M. davidsonii* (north), *M. davidsonii* (south), *M. fasciculatus* var. *catalinensis*, *M. fasciculatus* var. *nesioticus*, *M. fasciculatus* var. *nuttallii*, *M. involucratus*, *M. lucianus*, *M. marrubioides*, *M. mendocinensis*, *M. orbiculatus*, *M. palmeri,* "Shasta", and "Vandenberg".

The following three sets of species form exclusive clades with 100% BS in both trees but the species within them are not monophyletic: (1) *Malacothamnus arcuatus* and *M. hallii*; (2) *Malacothamnus fremontii* s.s., *M. helleri*, and *M. howellii*; and (3) *Malacothamnus jonesii* s.s., *M. gracilis*, and *M. niveus*.

Several morphological groupings from Volume 1 (Morse 2023) are not monophyletic in the full dataset without hybrids and are generally in clades with some samples from geographically adjacent groups. The majority of *Malacothamnus enigmaticus* is in a single clade (BS = 91) including three samples from other morphological groups, one a possible hybrid with *M. enigmaticus* (M063a) and one part of the morphologically similar "Baja East" group. Most of *M. densiflorus* var. *viscidus* is in a single clade (BS = 100). *Malacothamnus densiflorus* var. *densiflorus* is in three clades based on region. Santa Ana Mountains plants of *M. d.* var. *densiflorus* form a clade (BS = 99), which is a poorly supported sister to *M. d.* var. *viscidus*. San Jacinto Mountains plants of *M. d.* var. *densiflorus* are within a clade of eastern *M. fasciculatus* var. *laxiflorus*, which they are geographically adjacent to. These San Jacinto Mountains *M. densiflorus* are not monophyletic in the tree of the full dataset without hybrids but are a well-supported clade (BS = 100) in the reduced dataset tree. The remaining *M. d.* var. *densiflorus*, all from San Diego County, form the majority of a clade (BS = 96) with samples from five other geographically nearby morphological groups. The majority of *M. fasciculatus* s.s., *M. foliosus* sensu Bates, "*M. fasciculatus*/*foliosus* intermediates", and "Baja East/fasciculatus intermediates" form a clade (BS = 59), the crown of which is dominated by *M. foliosus* and the stem of which is dominated by *M. fasciculatus*. "Baja East" is scattered within the *M. densiflorus* var. *densiflorus* (San Diego County) and *M. enigmaticus* clades. *Malacothamnus fasciculatus* var *laxiflorus* is polyphyletic and split into two geographically cohesive clades. The eastern *M. f.* var. *laxiflorus* clade (BS = 87 in full dataset without hybrids and 95 in reduced dataset) is sister to *M. davidsonii* (south), with one sample of *M. f.* var. *laxiflorus* sister to both of these clades in the tree from the full dataset without hybrids. The western *M. f.* var. *laxiflorus* clade from both the full dataset without hybrids (BS = 79) and the reduced dataset (BS = 99) contains subclades of both *M. f.* var. *catalinensis* and *M. f.* var. *nesioticus*, which include all samples of each. Additionally, *M. marrubioides* is included within the clade of western *M. fasciculatus* var. *laxiflorus* in the full dataset without hybrids.

It is notable that branch lengths for *Malacothamnus* samples were much shorter than most of the outgroups with the only exception being *Iliamna*, which also has a fire-following life history (Fig. 7). These shorter branch lengths can be interpreted as showing less phylogenetic change relative to longer branches, which is often tied to more recently evolved lineages. Considering the fire-following nature of both *Malacothamnus* and *Iliamna* where plants may remain dormant in the soil seed bank for 100+ years without reproducing, these short branch lengths could also be interpreted as having a slower rate of phylogenetic change relative to plants that reproduce in all or most years. This hypothesis would need to be tested in a time-calibrated tree with a much higher sampling of outgroup taxa than included in this study. A further study with better sampling could ideally resolve some of the relationships between outgroup taxa as well, which are poorly supported and variable in placement in this study.

ML analysis of the full dataset with hybrids (Fig. S2) is similar to the dataset without hybrids but does have some topological changes that may provide additional clues about taxon relationships. Most putative hybrids fall out into clades with one of the putative parent taxa. Two samples with unknown parents fall out into or sister to a possible parental clade with 100% BS. One of these, a morphologically ambiguous specimen from California Botanic Garden (M385) is sister to *Malacothamnus clementinus*, which no longer occurs at the garden but grew there in the past. The other is an ambiguous specimen from Santa Cruz, CA (M173), which comes out in the *M. fasciculatus* var. *nesioticus* clade in a subclade with a Berkeley Botanic Garden specimen (UCBG_65_1187) that moved between the *M. fasciculatus* var. *nesioticus* clade and the *M. lucianus* clade in exploratory ML analyses.

One of the monophyletic taxa from other ML analyses is affected by the addition of hybrids. All *Malacothamnus fasciculatus* var. *nuttallii* samples form a well-supported clade when putative hybrids are excluded but forms two clades (one well-supported and one poorly supported) when putative hybrids are included. One of these clades is included in a larger clade with both “Vandenberg” and putative hybrids of “Vandenberg” and *M. fasciculatus* var. *nuttallii*. The remaining *M. fasciculatus* var. *nuttallii* is included in a larger clade with one *M. orbiculatus* and some likely hybrids of *M. orbiculatus* with possibly *M. fasciculatus* var. *laxiflorus* as a parent taxon. This clade is sister to *M. orbiculatus* with which *M. fasciculatus* var. *nuttallii* appears to hybridize with where their ranges meet.

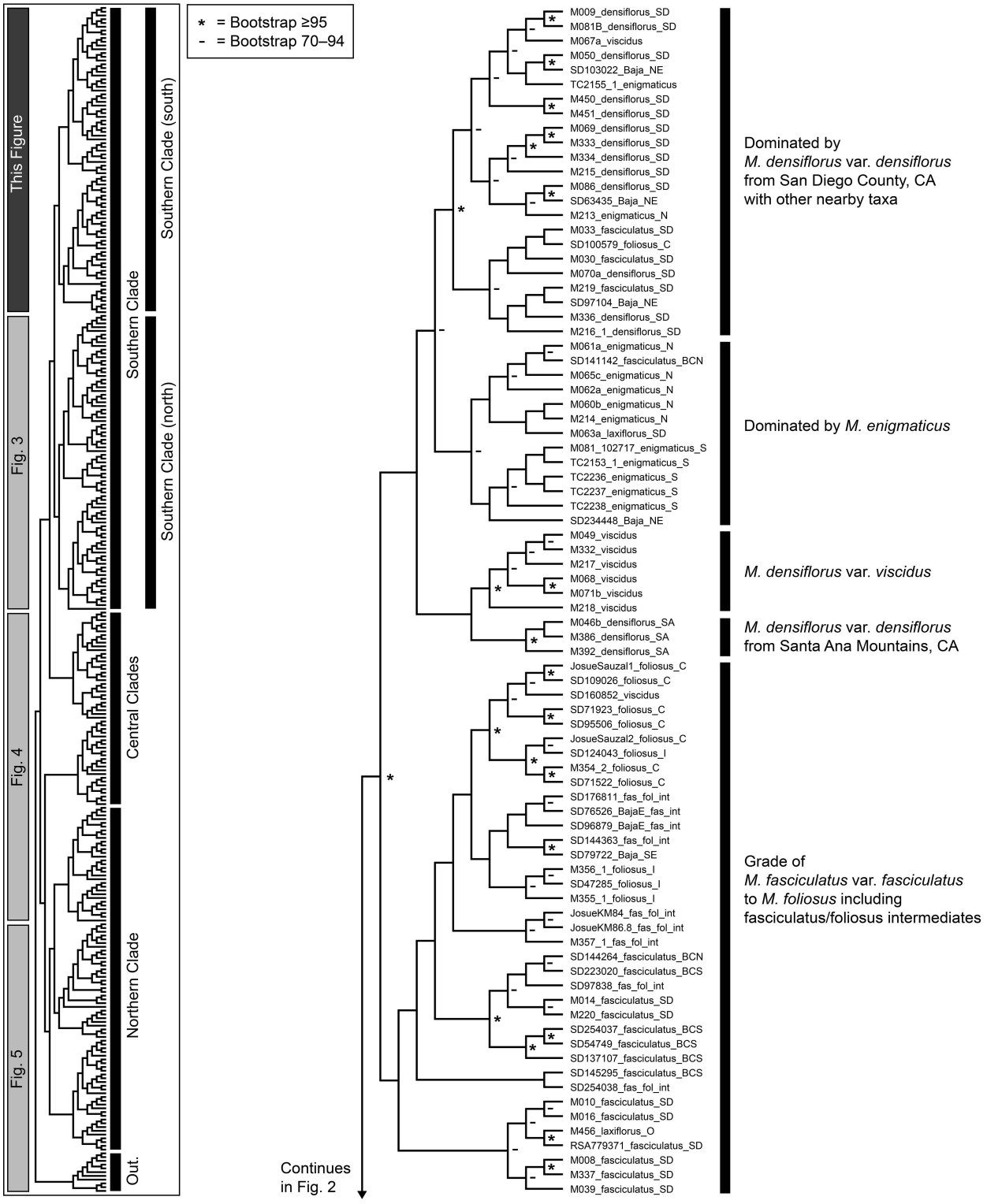

Figure 2. Consensus ML tree of full alignment without hybrids. Full tree on left. Part 1 of 4 enlarged on right. Clades representing morphological and geographic groupings are labeled.

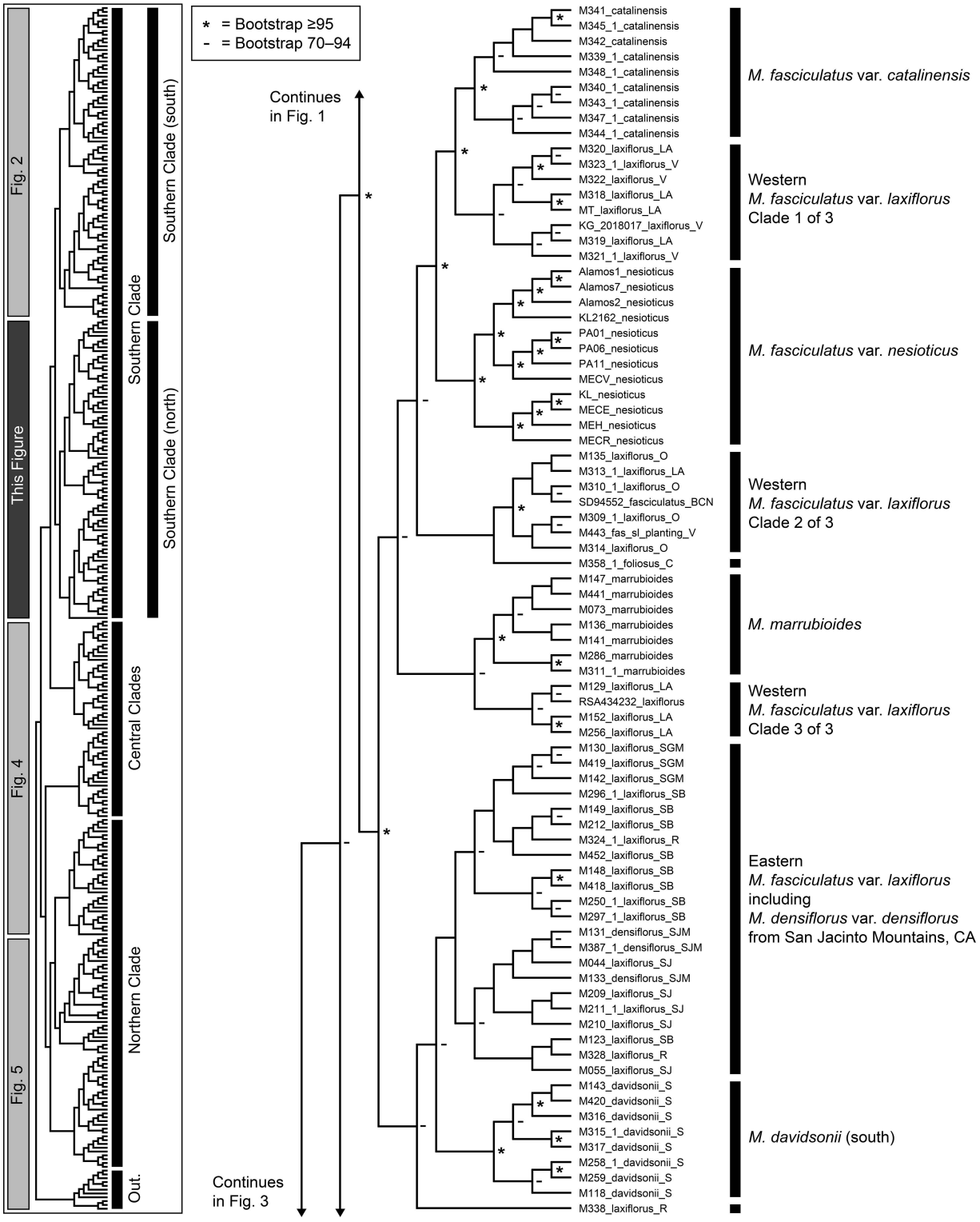

Figure 3. Consensus ML tree of full alignment without hybrids. Full tree on left. Part 2 of 4 enlarged on right. Clades representing morphological and geographic groupings are labeled.

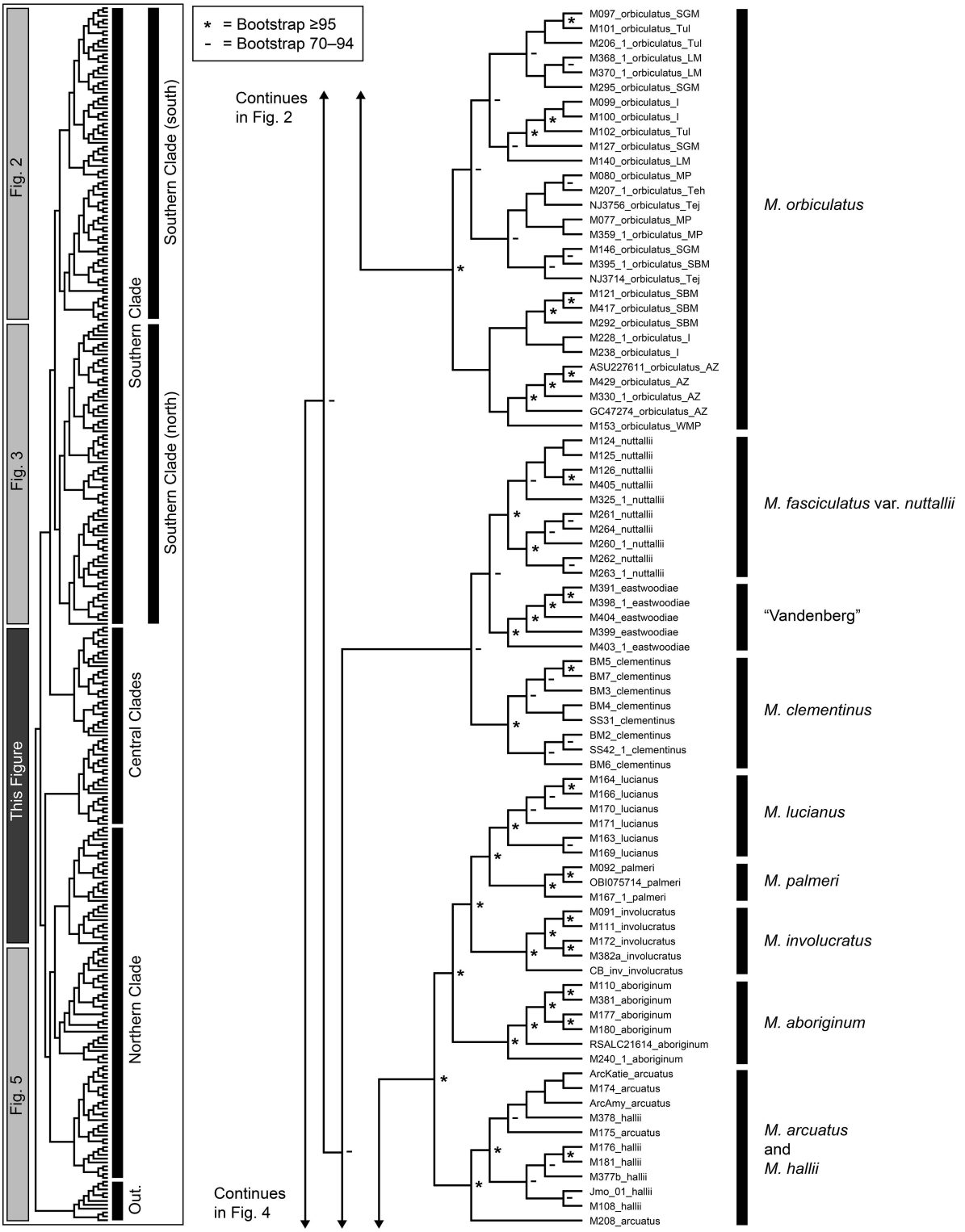

Figure 4. Consensus ML tree of full alignment without hybrids. Full tree on left. Part 3 of 4 enlarged on right. Clades representing morphological and geographic groupings are labeled.

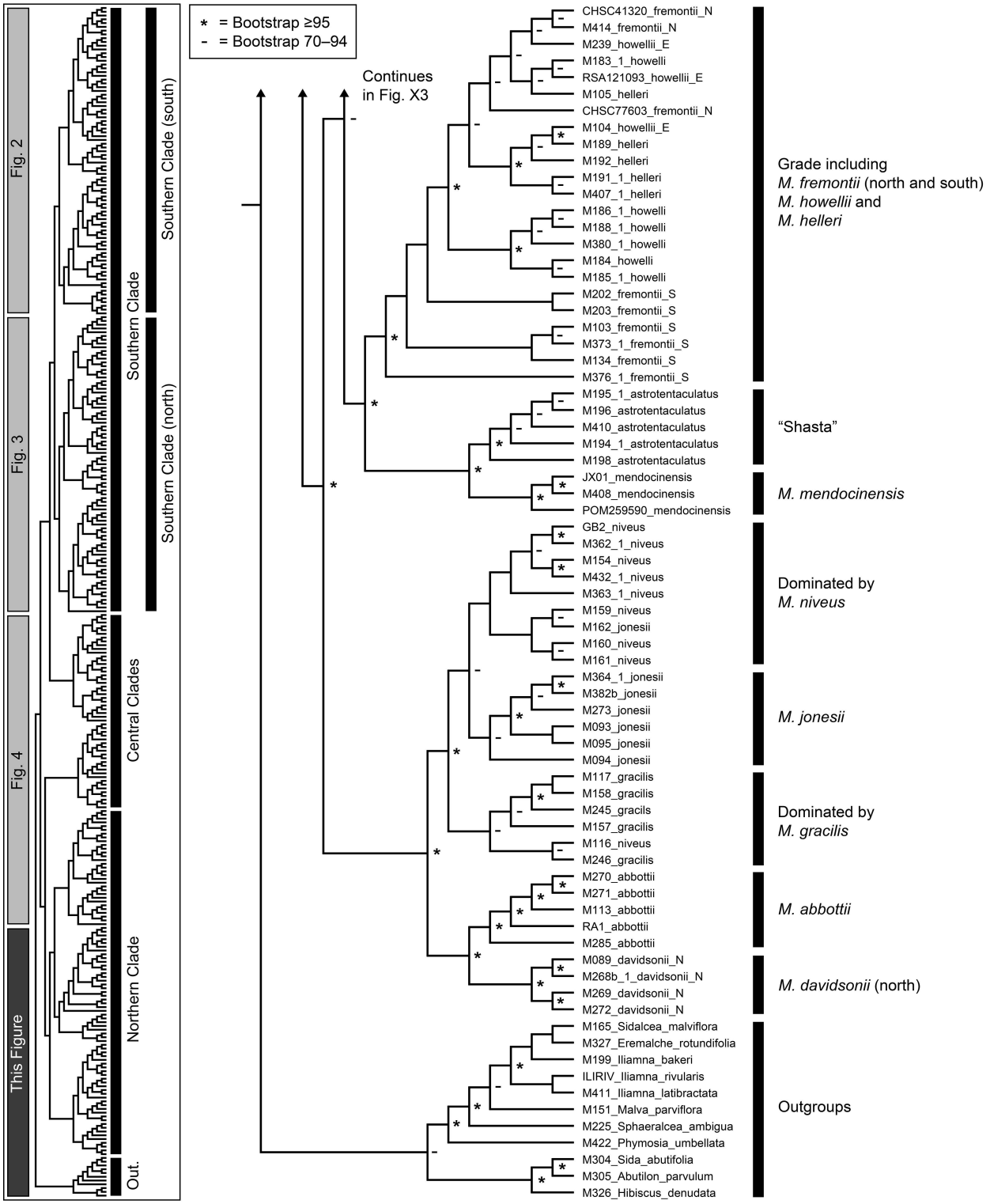

Figure 5. Consensus ML tree of full alignment without hybrids. Full tree on left. Part 4 of 4 enlarged on right. Clades representing morphological and geographic groupings are labeled.

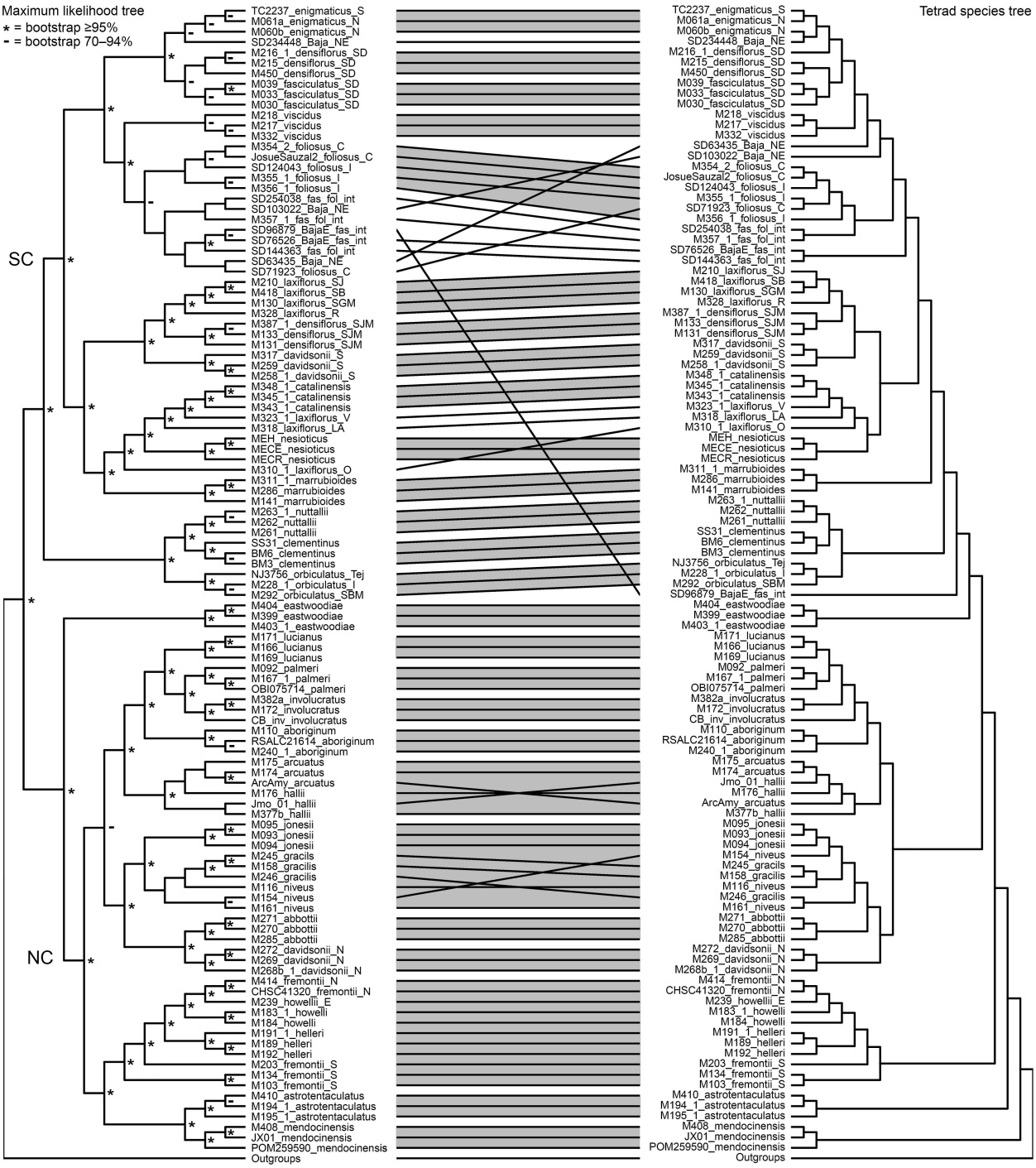

Figure 6. Tanglegram comparing the topology of ML (left) and Tetrad (right) consensus trees of the reduced dataset. Grey shading is used to more easily visualize morphologically and geographically cohesive clades. SC = Southern Clade. NC = Northern Clade.

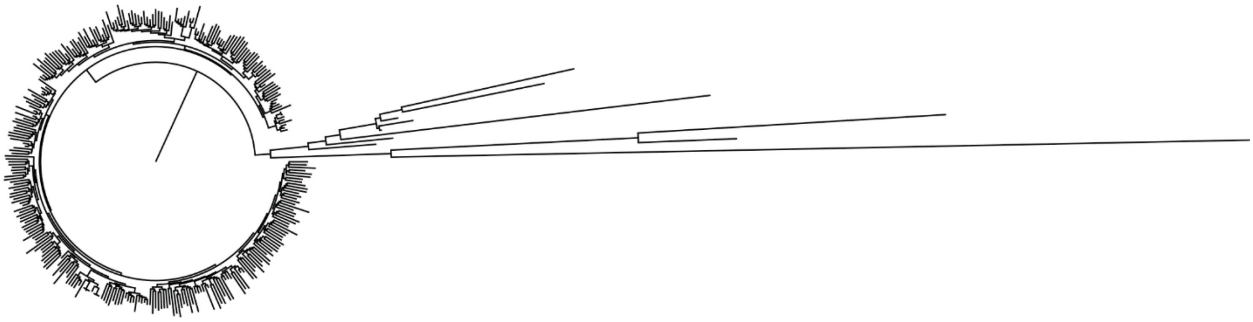

Figure 7. Consensus ML tree of full alignment without hybrids showing branch lengths without species labels. Note that the protruding branch lengths of the outgroups on the right are mostly much longer than the shorter branch lengths of *Malacothamnus* in the circle on the left. The three outgroups with shortest branch lengths are *Iliamna* species, which are fire-followers like *Malacothamnus*. The sample with the longest branch length is *Hibiscus denudatus*, which is the only sample included not in Malveae.

4.2 Tetrad Analyses

Tetrad analysis of the full dataset without hybrids yielded a partially resolved consensus tree (Fig. S3). Recovered clades are mostly consistent with the ML trees. Most samples that did not resolve in larger clades are from southern morphological groups that were generally not monophyletic in ML trees. Tetrad analysis of the reduced dataset has increased resolution and again recovered clades are mostly consistent with the ML trees as seen in a tanglegram comparing ML and Tetrad trees (Fig. 6). The starkest contrast in the tanglegram is SD96879 (a Baja East/fasciculatus intermediate). This conflict is likely at least partially due to missing data as this sample had the least number of loci of any *Malacothamnus* sample included in the analysis. Other major differences follow. In the ML tree, *M. mendocinensis* is sister to "Shasta" whereas, in the Tetrad tree, *M. mendocinensis* is sister to the full genus. In the ML tree, northern *M. davidsonii* is sister to *M. abbottii* whereas, in the Tetrad tree, northern *M. davidsonii* is sister to a clade of *M. jonesii* sensu Bates (*M. jonesii*, *M. gracilis*, and *M. niveus*) plus *M. abbottii*. In the ML tree, *M. palmeri* is sister to *M. involucratus* whereas, in the Tetrad tree, *M. palmeri* is sister to *M. lucianus*. In the ML tree, "Vandenberg" is sister to the Northern Clade of *Malacothamnus* taxa whereas, in the Tetrad tree, "Vandenberg" is sister to a clade including both the Southern Clade of *Malacothamnus* taxa and some geographically central taxa. In the ML tree, *M. marrubioides* is sister to a clade of *M. fasciculatus* var. *nesioticus*, *M. f.* var. *catalinensis*, and western *M. f.* var. *laxiflorus* whereas, in the Tetrad tree, *M. marrubioides* is sister to the rest of the Southern Clade. In the ML tree, *M. densiflorus* var. *viscidus* is sister to samples of other taxa from Baja California whereas, in the Tetrad tree, *M. densiflorus* var. *viscidus* is sister to the samples of other taxa from mostly San Diego County. *Malacothamnus densiflorus* var. *viscidus* occurs mostly in San Diego County near the border of Baja California.

4.3 Geographic Signal in Analyses

ML analysis of the full genus shows clear geographic signal as seen in a pruned phylogeny paired with a map of specimens analyzed (Fig. 8). Most clades corresponding to morphological groups are sister to geographically adjacent morphological groups. Most morphological groups can be assigned to a larger northern or southern clade. Samples from morphological groups that occur in intermediate areas ("Vandenberg", *M. fasciculatus* var. *nuttallii*, and *M. orbiculatus*) plus *M. clementinus* from San Clemente Island are placed in clades that fall between the northern and southern clades. These clades from intermediate regions are inconsistent in placement in trees, sometimes being a single clade sister to the northern or southern clade and sometimes being two clades with one sister to each the northern and southern clade.

The northern clade always includes *Malacothamnus abbottii*, *M. aboriginum*, *M. arcuatus*, *M. davidsonii* (north), *M. fremontii* s.s., *M. gracilis*, *M. hallii*, *M. helleri*, *M. howellii*, *M. involucratus*, *M. jonesii* s.s., *M. lucianus*, *M. mendocinensis*, *M. niveus*, *M. palmeri*, and "Shasta". Of these, *M. palmeri*, *M. involucratus*, and *M. lucianus* form a clade of geographically adjacent sister species, which supports the close relationships between them that was hypothesized by Kearney (1951, 1955) who recognized them as varieties of *M. palmeri* and by Bates (2015) who recognized them as a single species without varieties. Within the northern clade, there are three clades containing geographically adjacent morphological groupings that are not monophyletic. These are (1) *M. arcuatus* and *M. hallii*, which are part of *M. fasciculatus* sensu Bates; (2) *M. fremontii* s.s. (north and south), *M. helleri*, and *M. howellii*, which are part of *M. fremontii* sensu Bates; and (3) *M. jonesii* s.s., *M. gracilis*, and *M. niveus*, which together are *M. jonesii* sensu Bates.

The southern clade always includes *Malacothamnus davidsonii* (south), *M. densiflorus* (both varieties), *M. enigmaticus*, *M. fasciculatus* (all varieties except var. *nuttallii*), *M. foliosus* (coastal and inland), *M. marrubioides*, "Baja East", "Baja East/fasciculatus intermediates", and "M. fasciculatus/foliosus intermediates". Of these, only *M. davidsonii* (south), *M. marrubioides*, *M. fasciculatus* var. *catalinensis*, and *M. fasciculatus* var. *nesioticus* are always monophyletic, though the *M. f.* var. *catalinensis* clade is always included at the crown of a western *M. f.* var. *laxiflorus* clade and the other three occasionally are within *M. f.* var. *laxiflorus* clades. The remaining clades are generally dominated by a single morphological group, some only in a single clade and some in multiple clades. The scattered specimens throughout the larger southern clade that do not fall into clades consistent with their morphological and geographic groupings indicate possible geneflow and/or incomplete lineage sorting between adjacent groupings.

Clades intermediate to the northern and southern clades, both phylogenetically and mostly geographically, include *Malacothamnus clementinus*, *M. fasciculatus* var. *nuttallii*, and *M. orbiculatus*, and "Vandenberg". These taxa are all generally monophyletic, though *M.*

fasciculatus var. *nuttallii* is sometimes split into two clades that are sister to "Vandenberg" and *M. orbiculatus*, both of which *M. f.* var. *nuttallii* appears to hybridize with.

That *M. clementinus* falls into the intermediate clades is interesting from a geographic standpoint as it seems like somewhat of an outlier in Figure 8. It is the yellow island point mostly surrounded by green points. Morphologically, this makes some sense though as *M. clementinus* is most morphologically similar to some plants of *M. orbiculatus*. That these two species have a relatively recent common ancestor and that *M. clementinus* diverged as a result of being isolated on an island is perfectly plausible.

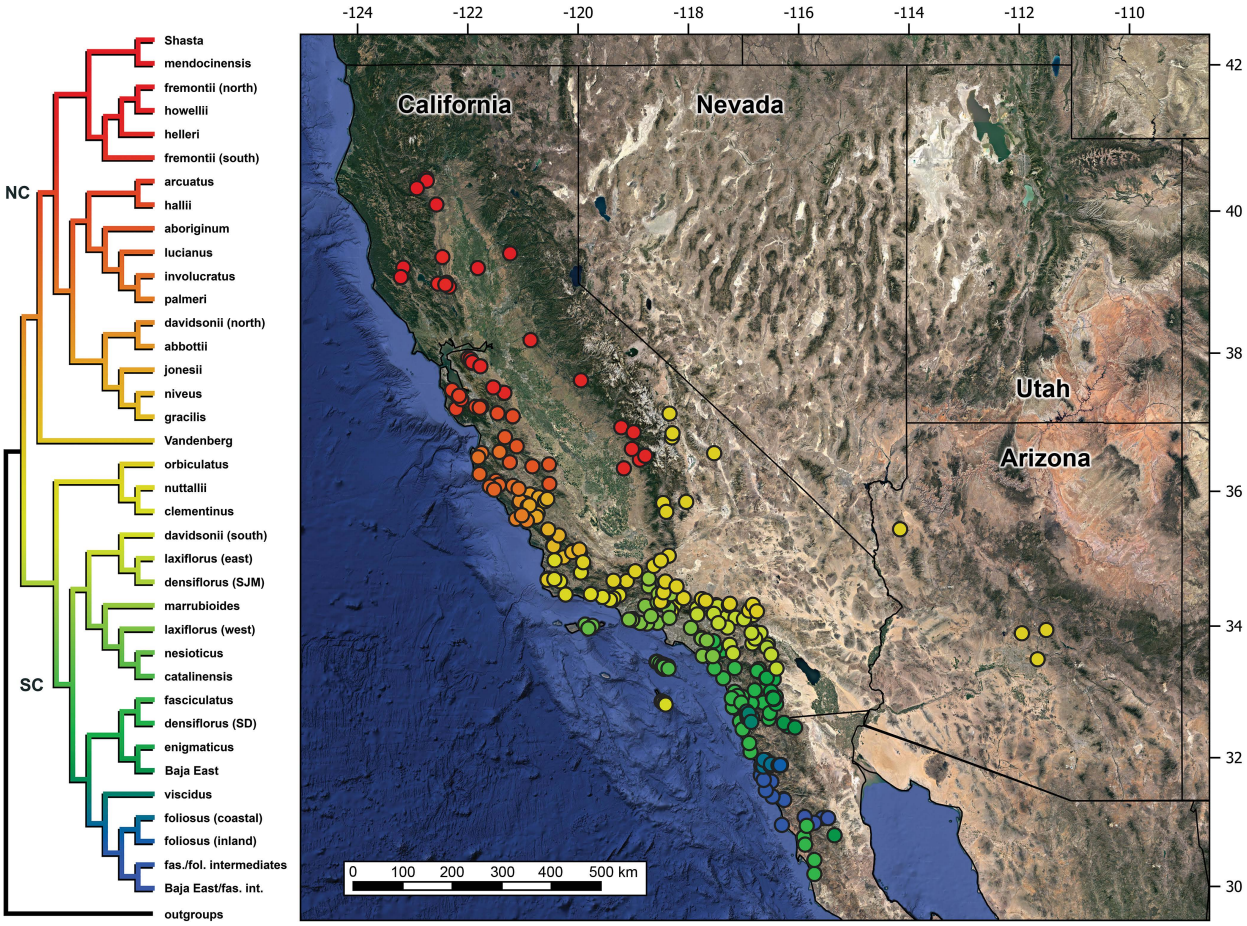

Figure 8. Pruned phylogeny of *Malacothamnus* labelled by morphological group and map of *Malacothamnus* specimens used in the full dataset without hybrids. Colors on the phylogeny tips correspond to mapped points. Phylogeny nodes are rotated to best illustrate the hypothesis that geographically adjacent taxa and clades may generally be more closely related to each other than those that are not geographically adjacent. SC = Southern Clade. NC = Northern Clade. Satellite imagery from Google Maps.

4.4 Phylogenetic Signal to Morphological Characters

To assess phylogenetic signal relative to morphological characters, I pair morphological character states from Volume 1 (Morse 2023) with a pruned phylogeny representing the morphological groups of Volume 1 (Fig. 9). While complex combinations of morphological characters do make for recognizable patterns between some closely related taxa in *Malacothamnus*, most morphological characters useful in distinguishing taxa are sporadic throughout the topology of the phylogenies. The overly broad species delimitations of Bates (2015) can be viewed as a summary of some morphological patterns in the genus and are included in Figure 9 to compare with individual characters. These patterns correspond to the phylogenetic relationships of some clades such as *M. palmeri* sensu Bates and *M. jonesii* sensu Bates, however, taxa included in *M. fasciculatus* sensu Bates and *M. fremontii* sensu Bates are spread throughout the phylogeny. Only two characters stand out as especially informative for a single clade: how corollas dry and inflorescence type.

Malacothamnus corollas generally dry closed after anthesis, however, a few taxa have corollas drying fully or partially open (Character 2 in Fig. 9). The *M. fremontii* clade (*M. fremontii*, *M. helleri*, and *M. howellii*) form the only clade with corollas generally drying fully open. One of the sister species to this clade, "Shasta", has corollas generally drying partially open, which indicates a possible apomorphy in a common ancestor or that gene flow between a member of the *M. fremontii* clade and "Shasta" may have led to an intermediate morphological state in "Shasta". Corollas drying partially open was also observed in apparent hybrids of *M. howellii* with *M. hallii*, which has corollas drying closed. Five other taxa had aberrant specimens with corollas drying partially to fully open, so this character state is not exclusive to the *M. fremontii* clade and "Shasta" but does provide a morphological character that usually differentiates these groups from the rest.

Inflorescence type varies on a spectrum from capitate to subcapitate to spike-like to panicle-like (Character 1 in Fig. 9). While spike-like and panicle-like inflorescences are scattered throughout the phylogeny, capitate to subcapitate inflorescences are almost exclusive to the *Malacothamnus palmeri* clade (*M. palmeri*, *M. involucratus*, and *M. lucianus*). This may indicate that the capitate to subcapitate inflorescence type was an apomorphy in a common ancestor of the *M. palmeri* clade. Within the *M. palmeri* clade, *M. involucratus* may also have spike-like inflorescences, sometimes on plants that also have subcapitate inflorescences. Outside of the *M. palmeri* clade, subcapitate inflorescences have been documented in aberrant plants of "Shasta" and *M. densiflorus* var. *densiflorus*, both of which generally have spike-like inflorescences. It is worth noting that while *M. densiflorus* var. *densiflorus* almost exclusively has spike-like inflorescences, I have documented plants with subcapitate and panicle-like inflorescences, each rare in single but separate populations of more typical plants. This, and a general tendency for many taxa to have spike-like to panicle-like inflorescence variation, indicates the potential for aberrant inflorescence types in possibly any species.

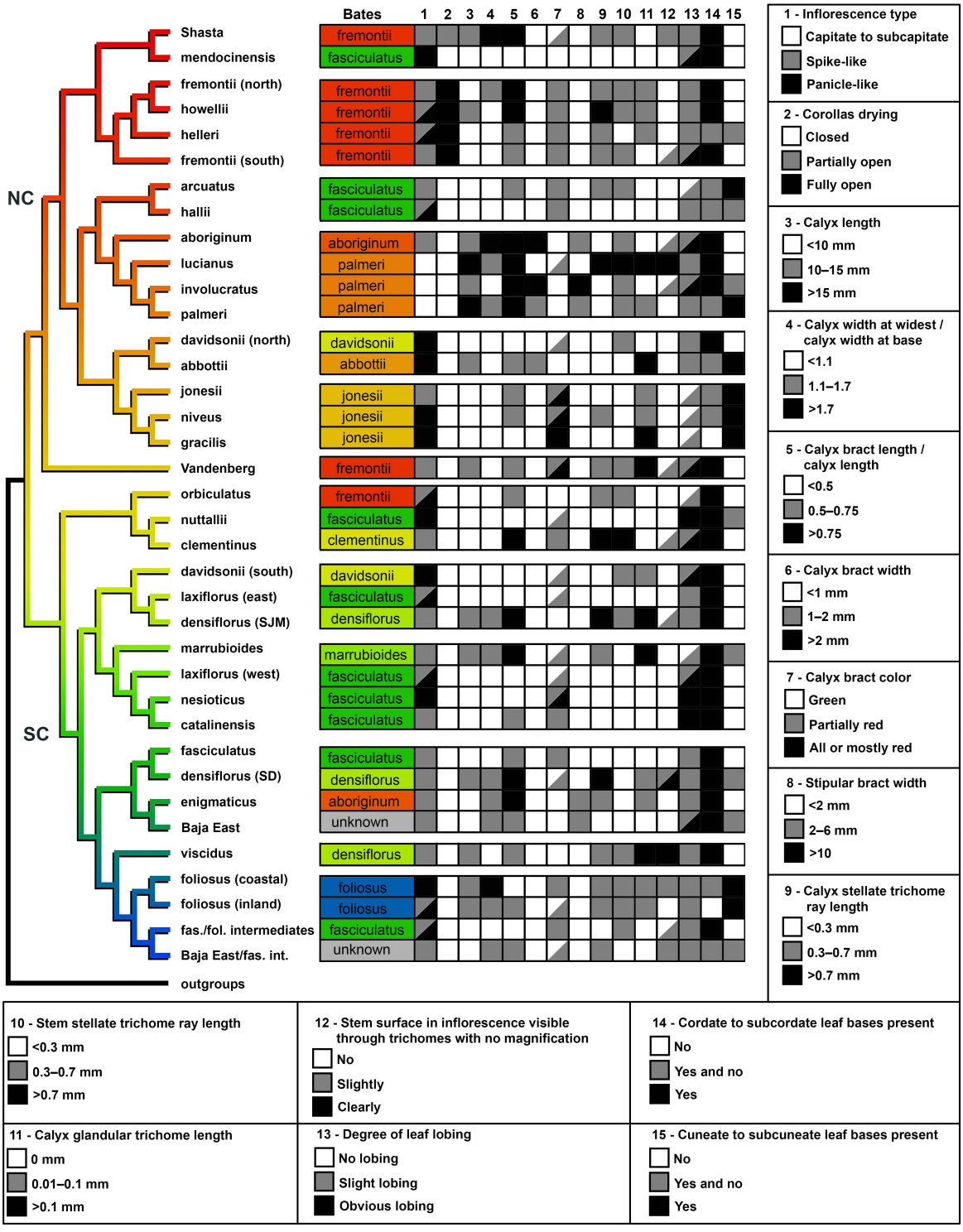

Figure 9. Fifteen morphological characters measured in Volume 1 mapped to a pruned phylogeny of *Malacothamnus*. Phylogeny coloration corresponds to (Fig. 8). "Bates" indicates which of the broadly defined morphological species of Bates (2015) each phylogeny tip is classified as by Bates. Coloration of Bates's species matches the tip containing the type specimen of each. SC = Southern Clade. NC = Northern Clade. Continuous variables use the mean value of each tip and are classified to a single category. Qualitative characters show all character values represented in >25% of specimens assessed, which may be displayed as two categories split diagonally in a single square.

4.5 Hybridization Insights

Hybridization in *Malacothamnus* is readily apparent based on field observations. Most *Malacothamnus* taxa are allopatric and morphologically distinct, however, morphological intermediate plants are often observed where the ranges of these taxa meet. The putative taxa involved in hybridization are usually obvious based on the presence of only two possible parent taxa within an area. In areas where more than two taxa come together, phylogenetic analyses are needed to confirm which taxa may have contributed to putative hybrids. ML analysis offers at least some level of evidence confirming likely hybridization. In most cases, putative hybrids resolved in or sister to clades of one of the expected parent taxa (Fig. S2). A dataset with less missing data and population-level rather than a broad geographic sampling is needed to better assess hybridization within the genus though.

Beyond a few insights gained on possible parental contributors to hybrids of plants in a cultivated setting, only one major insight into natural populations was found. Morphologically intermediate plants are common in some areas around Santa Clarita, CA, where the ranges of *Malacothamnus orbiculatus*, *M. marrubioides*, and *M. fasciculatus* var. *laxiflorus* come together. As hybridization is apparently common where *Malacothamnus* taxa come together, it is possible that all three taxa could contribute to some of the intermediates in this region. In ML analysis (Fig. S2), these intermediates were all included in the *M. orbiculatus* clade or with part of *M. fasciculatus* var. *nuttallii*, which was sister to the *M. orbiculatus* clade. While *M. fasciculatus* var. *nuttallii* is not immediately adjacent to this area, it is nearby and there are intermediates of *M. fasciculatus* var. *nuttallii* and *M. orbiculatus* not far away. Therefore, if a future researcher investigates the origin of the intermediate plants in the Santa Clarita area, they should include *M. f.* var. *nuttallii* in the sampling as a possible parent taxon. The fact that the intermediates from the Santa Clarita area did not fall out in either the *M. marrubioides* or *M. fasciculatus* var. *laxiflorus* clades is interesting but perhaps a misleading clue related to deficient sampling of both specimens and loci.

5 TAXON SUBSET ANALYSES

Based on the combination of morphological, geographic, and phylogenetic evidence from full genus analyses, the following morphological groups should unquestionably be treated at the rank of species: *Malacothamnus abbottii*, *M. aboriginum*, *M. clementinus*, *M. involucratus*, *M. lucianus*, *M. marrubioides*, *M. mendocinensis*, *M. orbiculatus*, *M. palmeri,* "Shasta", and "Vandenberg". All 11 of these species are monophyletic and most specimens of each are morphologically distinct from all other species. Those few specimens that may be confused with other species occur within exclusive geographic ranges that do not contain other species they may be confused with. "Shasta" and "Vandenberg" are described as new species in Chapter 6.

To further assess the relationships of the remaining morphological groups, additional phylogenetic analyses were undertaken on subsets of these groups. In particular, neighbor-net analysis and MDS were used to assess relationships of smaller groupings outside the confines of a bifurcating tree. ML analysis was also run on the varieties of *Malacothamnus fasciculatus*. Additional analyses and discussions regarding the remaining morphological groups follow including formalization of taxonomic changes in some of these groups.

5.1 *Malacothamnus arcuatus* and *M. hallii*

Malacothamnus arcuatus and *M. hallii* are geographically adjacent and mostly morphologically distinct but appear to intergrade where the two species meet (Fig. 10). See Volume 1 (Morse 2023) for the morphological analyses. Neither of these taxa is monophyletic but together they form a single clade in ML (Fig. 4) and Tetrad analyses (Fig. 6). Neighbor-net analysis and MDS were performed on just *M. arcuatus* and *M. hallii* to further explore their relationship to one another.

Neighbor-net analysis of *Malacothamnus arcuatus* and *M. hallii* shows both species to be mostly separated except for one sample assigned to *M. hallii* (M378) appearing to be possibly intermediate (Fig. 11). This sample is notable in having the least number of recovered loci of those samples included in this analysis. Additionally, both M377b (*M. hallii* in this analysis) and M378 were collected only ~1 km apart in what is possibly a transition zone between *M. arcuatus* and *M. hallii*. So, it is unclear whether M378 is actually an intermediate or its placement in the network is just an artifact of missing data.

MDS of *Malacothamnus arcuatus* and *M. hallii* possibly indicates two species with intermediates but sampling is too poor to interpret the plot (Fig. 12) with any certainty. Two of the five *M. arcuatus* samples are strong outliers relative to the rest. Specimen M378, which plots ambiguously in Figure 11, plots with the geographically nearby M377b here. Both M377b and M378 are from the transition zone between the two species, which suggests a higher possibility of intermediacy than the rest of the samples. The conflict between M377b and M378

plotting with samples of *M. arcuatus* in MDS and M377b plotting with *M. hallii* in neighbor-net analyses, indicates possible phylogenetic evidence for intergradation where the ranges of the two species meet, but analyses with higher sampling would be needed to confirm.

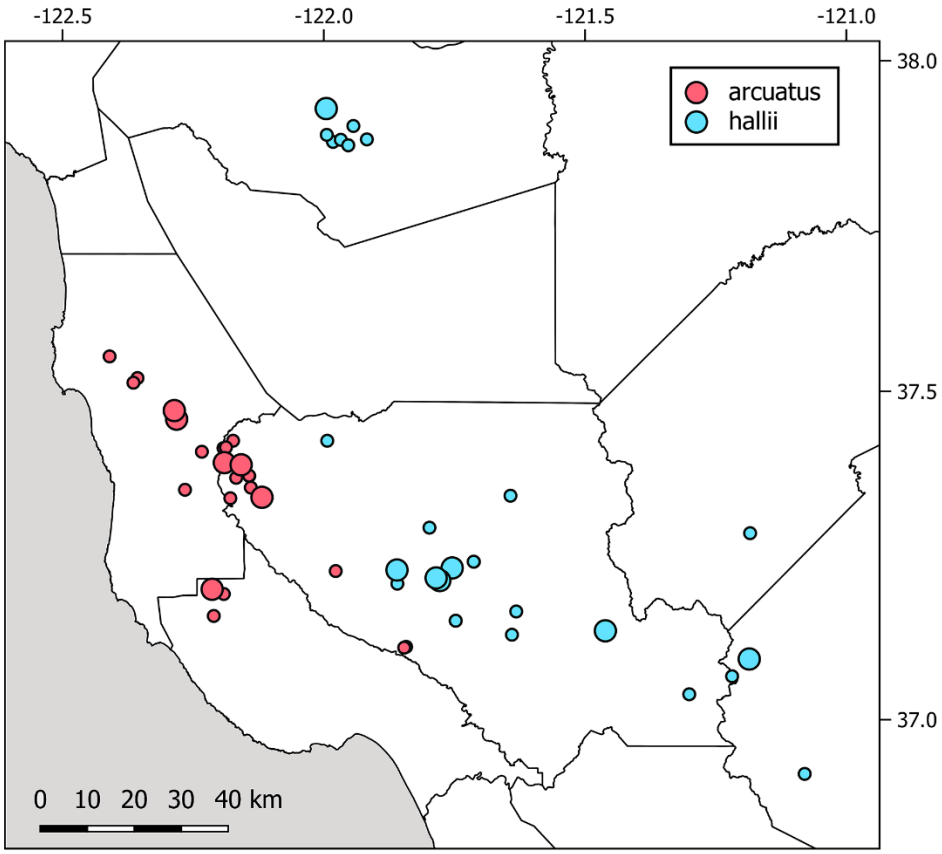

Figure 10. Map of *Malacothamnus arcuatus* and *M. hallii.* Smaller circles indicate specimens measured in morphological analyses. Larger circles indicate specimens used in phylogenetic analyses. Black lines indicate boundaries of California counties.

As *Malacothamnus arcuatus* and *M. hallii* can be mostly distinguished morphologically, are mostly distinct in neighbor-net analysis, and are geographically distinct outside of a zone of intermediates, I believe they should be recognized as different taxa despite forming a single clade and neither being monophyletic in ML analyses. While treating *M. arcuatus* and *M. hallii* as two species may be justifiable, they are, however, morphologically similar and may represent a divergence of two geographically adjacent lineages currently in progress. Further research with increased sampling could possibly confirm or refute this. In the meantime, I recognize *M. hallii* as a variety of *M. arcuatus*, which allows more ambiguous specimens from the transition zone between them to be assigned to *M. arcuatus* without identifying to variety.

The only valid basionym at the variety rank within the *Malacothamnus hallii* morphological group is *Sphaeralcea fasciculata* var. *elmeri*, which has never been published in the genus *Malacothamnus* and thus necessitates a new combination.

MALACOTHAMNUS ARCUATUS (Greene) Greene var. ELMERI (Jeps.) Morse, comb. nov. *Sphaeralcea fasciculata* (Nutt. ex Torr. & A.Gray) Arthur var. *elmeri* Jeps., Fl. Calif. [Jepson] 2: 501. 1936. *Malvastrum hallii* Eastw., Leafl. W. Bot. 1: 216. 1936. *Malacothamnus hallii* (Eastw.) Kearney, Leafl. W. Bot. 6: 134. 1951. TYPE: U.S.A. California: Contra Costa County, Mount Diablo, May 1903, *A. D. E. Elmer 4395* (Holotype: JEPS2868 [photo!], isotypes: DS65160 [photo!], GH00058121 [photo!], NY00222084 [photo!], POM61908!). Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325441-1

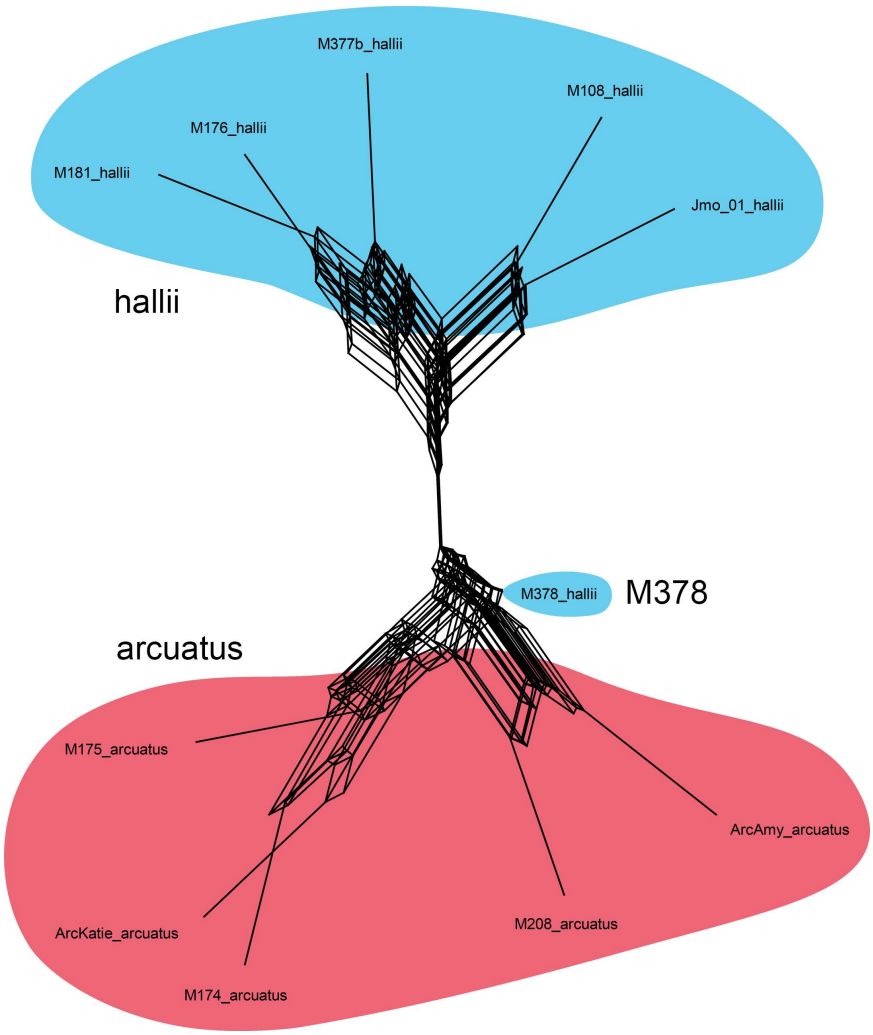

Figure 11. Neighbor-net analysis of *Malacothamnus arcuatus* and *M. hallii.*

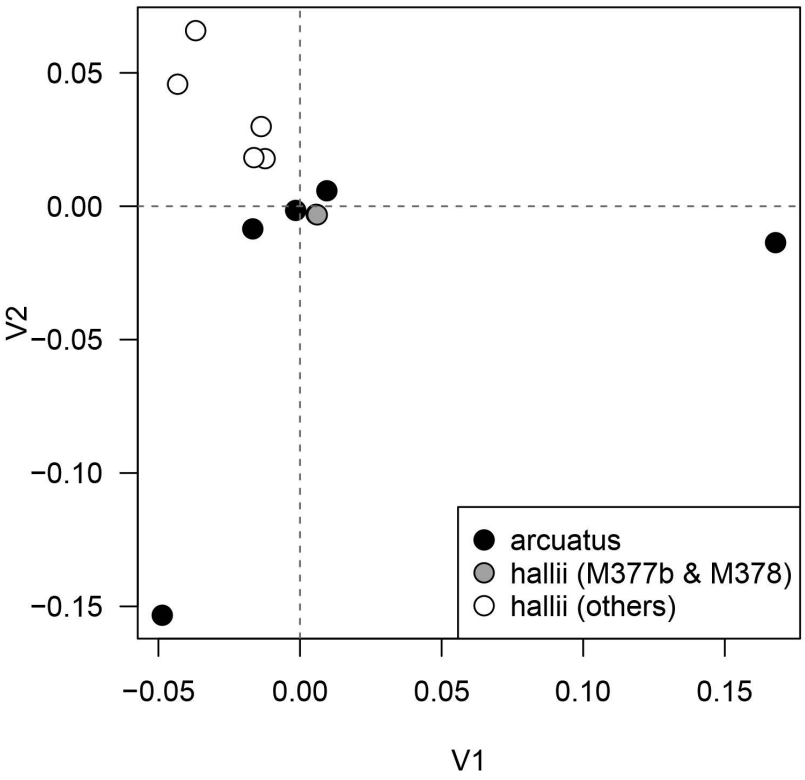

Figure 12. MDS of *Malacothamnus arcuatus* and *M. hallii*. Specimens M377b and M378, shown in grey, plot on top of each other.

5.2 *Malacothamnus fremontii, M. howellii, and M. helleri*

The morphological groupings *Malacothamnus fremontii* s.s. (north and south), *M. howellii*, and *M. helleri* are united in the morphological character of being the only groups where the corolla almost always dries fully open, rather than closed or partially open. The northern *M. fremontii* s.s. group is geographically closest to *M. howellii* and *M. helleri* with the northernmost occurrence just south of the "Shasta" group (Fig. 13). The southern *M. fremontii* s.s. group is geographically separated from the northern group by *M. howellii*. Of these four groups, *M. helleri* was the only one found to be both morphologically and geographically distinct from the rest. Southern *M. fremontii* s.s. and *M. howellii* formed generally distinguishable morphological groups in PCAs of morphological traits with the northern group of *M. fremontii* s.s. plotting as possibly morphologically intermediate between these groups. However, further examination of additional specimens that were not included in morphological analyses shows many possible morphological intermediates from the eastern region of *M. howellii* as well, which is geographically between the northern and southern *M. fremontii* s.s. regions. See Volume 1 (Morse 2023) for more details on the morphological analyses.

The four morphological groupings *Malacothamnus fremontii* s.s. (north and south), *M. howellii*, and *M. helleri* always form a single clade in ML analyses of molecular data with the groupings forming somewhat of a grade within. Within this grade, *Malacothamnus fremontii* s.s. (south) is always at the stem of the grade, *M. helleri* frequently resolves as monophyletic and is usually separated from *M. fremontii* s.s. (south) by some samples of *M. howellii*, and the remaining *M. howellii* and *M. fremontii* s.s. (north) form the crown of the grade. Neighbor-net analysis and MDS were performed on just *Malacothamnus fremontii* s.s. (north and south), *M. howellii*, and *M. helleri* to further explore their relationships to one another.

Neighbor-net analysis shows the four morphological groups to form clusters in the network (Fig. 14). While *M. fremontii* s.s. (south), *M. helleri*, and *M. howellii* form relatively distinct clusters, two samples of the northern *M. fremontii* s.s. appear to be possibly intermediate between *M. helleri* and *M. howellii* and the third (CHSC77603) appears to be possibly intermediate between all groups. CHSC77603 and RSA121093 have less than half the loci recovered of the other included samples, which may at least partially account for the placement of CHSC77603.

MDS of *Malacothamnus fremontii* s.s. (north and south), *M. helleri*, and *M. howellii* shows some clustering of the four morphological groups but no clear separation of any (Fig. 15).

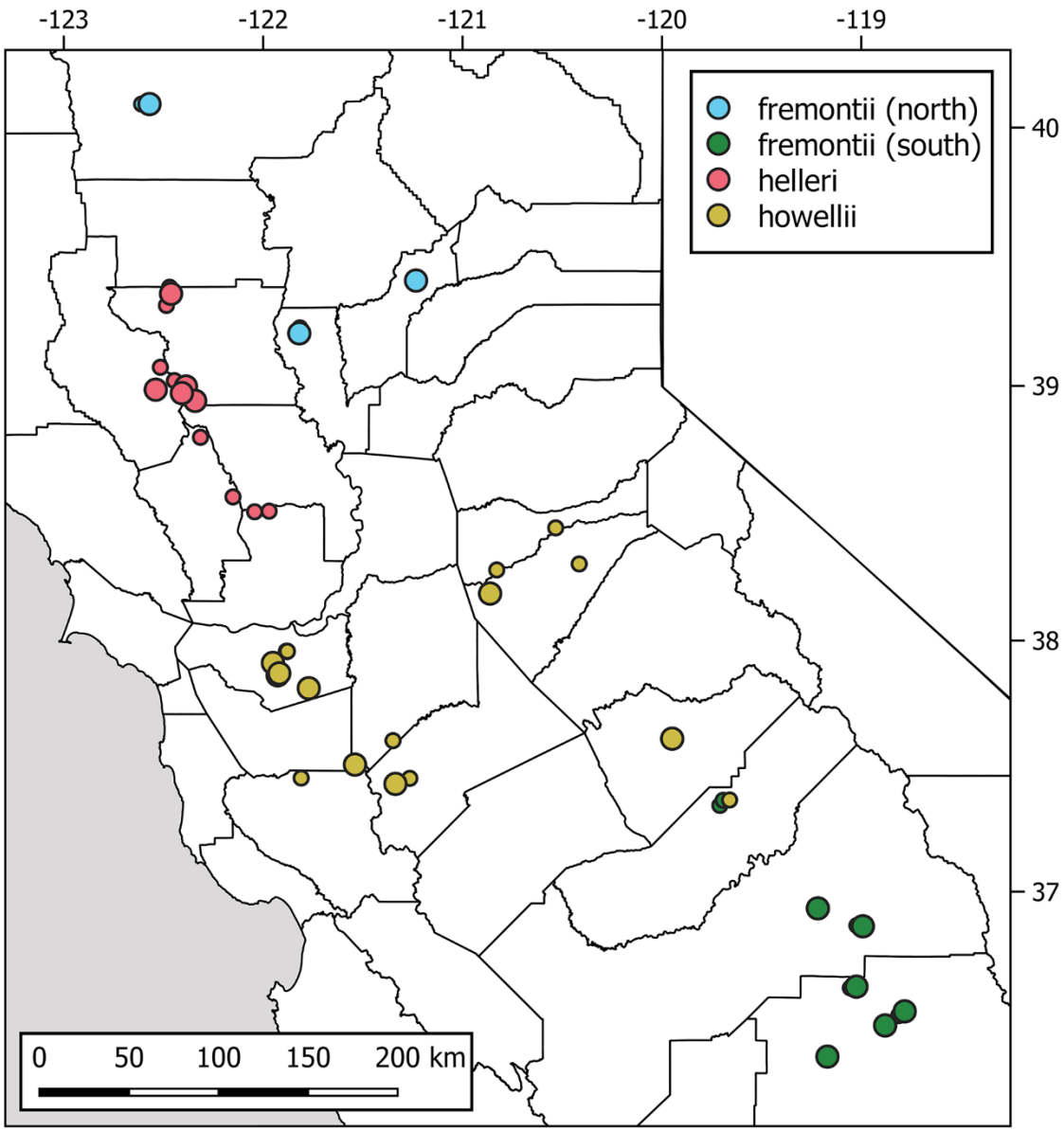

Figure 13. Map of *Malacothamnus fremontii* s.s. (north and south), *M. helleri*, and *M. howellii*.. Smaller circles indicate specimens measured in morphological analyses but not used in phylogenetic analyses. Larger circles indicate specimens used in phylogenetic analyses. Black lines indicate boundaries of California counties.

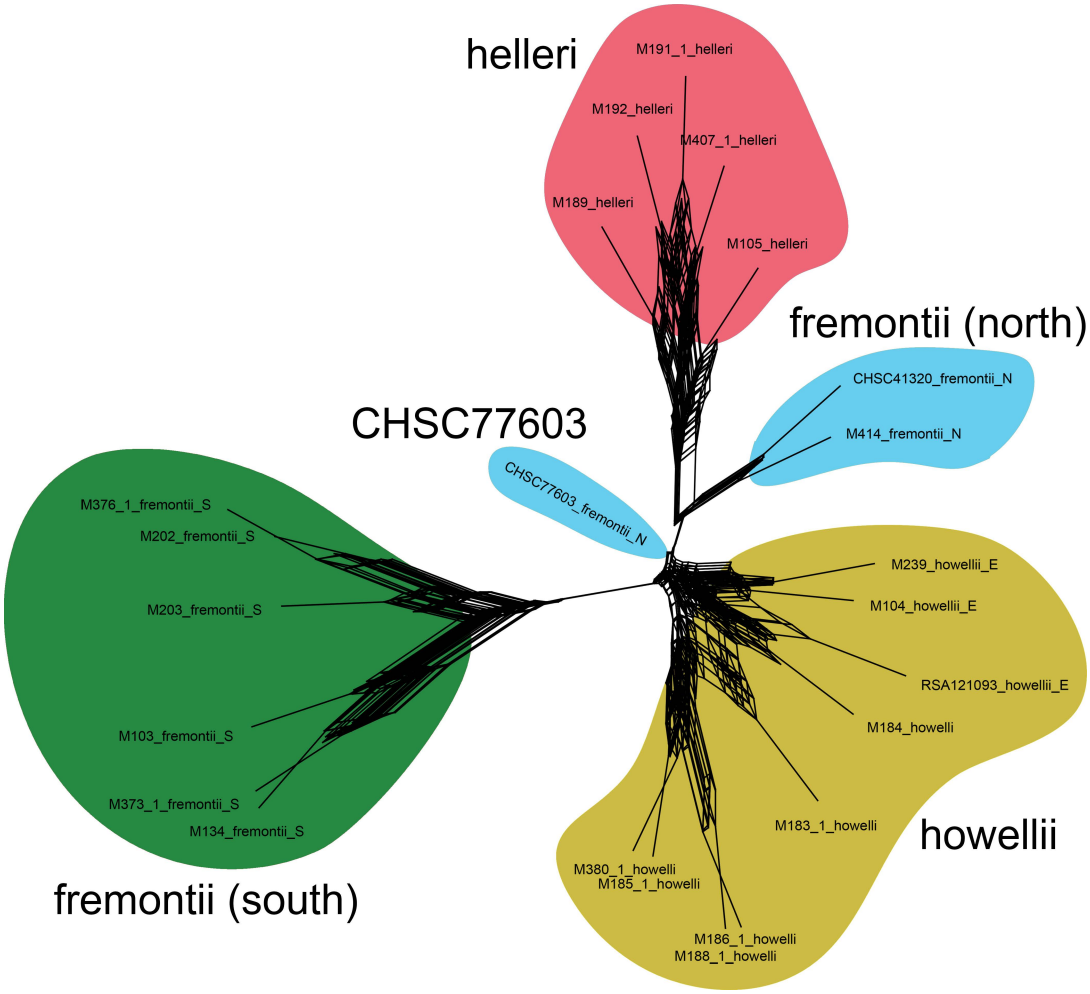

Figure 14. Neighbor-net analysis of *Malacothamnus fremontii* s.s. (north and south), *M. helleri*, and *M. howellii*.

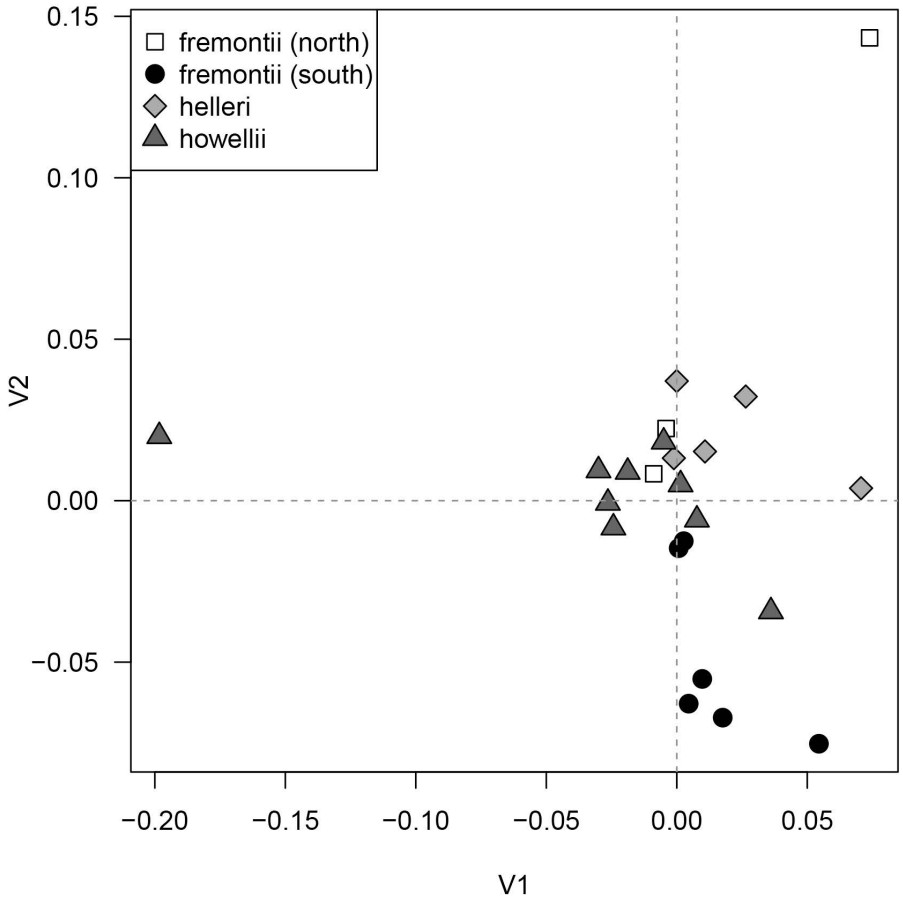

Figure 15. MDS of *Malacothamnus fremontii* s.s. (north and south), *M. helleri*, and *M. howellii*.

Neighbor-net analysis indicates *M. fremontii* s.s. (south*), M. howellii*, and *M. helleri* may be separate lineages, however *M. fremontii* (north) appears to be possibly intermediate between *M. howellii* and *M. helleri* (Fig. 14). Similar patterns are seen in MDS (Fig. 15).

Despite the northern *Malacothamnus fremontii* s.s. group occurring in a geographically distinct area, its morphological and phylogenetic intermediacy makes it problematic for classification. Further sampling in the large gaps in the current known geographic distribution of both northern and southern *M. fremontii* s.s. could possibly clarify how best to recognize the northern group. As the northern *M. fremontii* s.s. appears to be morphologically intermediate between southern *M. fremontii* s.s. and *M. howellii*, and possibly phylogenetically intermediate between *M. howellii* and *M. helleri*, it seems best to treat all four of these morphological groupings within a single species until a future researcher can clarify the relationships between them. As there are many plants that appear both morphologically and geographically intermediate between *M. howellii* and the northern and southern regions of *M. fremontii* s.s., and as I have had difficulties devising a key to separate them reliably using morphology, I treat

M. howellii as a synonym of *M. fremontii* s.s., recognizing that further research could very likely justify its recognition as a distinct or relatively distinct taxon.

As *Malacothamnus helleri* can be morphologically distinguished from the rest of the *M. fremontii* clade, is generally monophyletic within this clade, and has a distinct geographic range, I treat it as a variety of *M. fremontii*. The oldest valid basionym at the variety rank within the *M. helleri* morphological group is *Sphaeralcea fremontii* var. *exfibulosa*, which has never been published in the genus *Malacothamnus* and thus necessitates a new combination.

MALACOTHAMNUS FREMONTII (Torr. ex A.Gray) Greene var. EXFIBULOSUS (Jeps.) Morse, comb. nov.

Malvastrum helleri Eastw., Leafl. W. Bot. 1: 217 (Apr. 1936). *Sphaeralcea fremontii* var. *exfibulosa* Jeps., Fl. Calif. [Jepson] 2: 500 (Sep. 1936). *Malvastrum fremontii* var. *helleri* (Eastw.) McMinn, Man. Calif. Shrubs 341 (1939). *Malacothamnus helleri* (Eastw.) Kearney, Leafl. W. Bot. 6: 124 (1951). TYPE: U.S.A. California: [western Yolo County], on the lower terrace of Putah Creek near Winters, 11 June 1934 [note on specimen says actually collected on June 18 according to Jepson's field notebook], W. L. Jepson 16741 (Holotype: JEPS2869 [photo!], isotype: RSA279363!).

Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325442-1

5.3 *Malacothamnus jonesii* sensu Bates

Malacothamnus jonesii s.s., *M. gracilis,* and *M. niveus* (collectively *M. jonesii* sensu Bates) possibly represent three species in the process of diverging from one. Both *M. jonesii* s.s. and *M. gracilis* are geographically adjacent to and appear to intergrade with *M. niveus* where their ranges meet (Fig. 16). All three species are morphologically similar to one another but usually easily distinguishable based on inflorescence and trichome characters. See Volume 1 (Morse 2023) for more details on the morphological analyses.

These three morphological groupings always form a single clade in ML analyses of RAD-seq data. Within this clade, *M. gracilis* and *M. jonesii* s.s. are often monophyletic or mostly monophyletic whereas *M. niveus* is occasionally monophyletic but often split within the larger clade. However, unlike the *M. arcuatus/hallii* clade and *M. fremontii* clade, the species in the *M. jonesii* clade do not form distinct clusters in neighbor-net analysis or MDS (Fig. 17, Fig. 18, and Fig. 19), possibly indicating that they are less diverged.

I have observed at least partial divergence of flowering time between *M. jonesii* s.s., *M. gracilis*, and *M. niveus*. *Malacothamnus jonesii* s.s. mostly finishes flowering before *M. niveus* starts and *M. gracilis* generally flowers later and longer than *M. niveus*. Whether or not this phenological divergence would hold up in a common garden needs to be tested.

Malacothamnus jonesii s.s., *M. gracilis,* and *M. niveus* could arguably be treated as separate species that are in the process of diverging or as varieties of *M. jonesii*. As they form three similar and geographically adjacent morphological groups that intergrade and as there is little evidence that each could be monophyletic, I treat them as varieties of *M. jonesii*, which allows intermediate specimens to be easily classified to the species rank. *Malacothamnus gracilis* has not been treated at the variety rank in the past and needs a rank change. *Malacothamnus niveus* has been treated as a variety in the past as *Malvastrum fremontii* var. *niveum* (Eastw.) McMinn and needs a new combination within *Malacothamnus jonesii*.

MALACOTHAMNUS JONESII (Munz) Kearney var. GRACILIS (Eastw.) K.Morse, comb. et stat. nov.
Malvastrum gracile Eastw., Leafl. W. Bot. 1: 219 (1936). *Malacothamnus gracilis* (Eastw.) Kearney, Leafl. W. Bot. 6: 130 (1951). TYPE: U.S.A. California: San Luis Obispo County, on road from Arroyo Grande to Huasna, 30 July 1927, *A. Eastwood 14996* (Holotype: CAS228727 [photo!], isotypes: CAS 228728 [photo!], F866403 [photo!], GH00052899 [photo!], K000659254 [photo!], NY00221824 [photo!], POM224487!, RSA77210!, US1678331 [photo!]).
Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325443-1

MALACOTHAMNUS JONESII (Munz) Kearney var. NIVEUS (Eastw.) K.Morse, comb. nov. *Malvastrum fragrans* Eastw., Leafl. W. Bot. 1: 218 (1936), nom. superfl., non *Malvastrum fragrans* A.Gray & Harv., Fl. Cap. (Harvey) 1: 159 (1860). *Malvastrum niveum* Eastw., Leafl. W. Bot. 1: 232 (1936). *Malvastrum fremontii* var. *niveum* (Eastw.) McMinn, Man. Calif. Shrubs 343 (1939). *Malacothamnus niveus* (Eastw.) Kearney, Leafl. W. Bot. 6: 123

(1951). TYPE: U.S.A. California: San Luis Obispo County, Santa Margarita, El Dorado School, 28 May 1933, M. E. Wall s.n. (Holotype: CAS204656!, isotypes: GH00052897 [photo!], K000659255 [photo!]).
Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325444-1

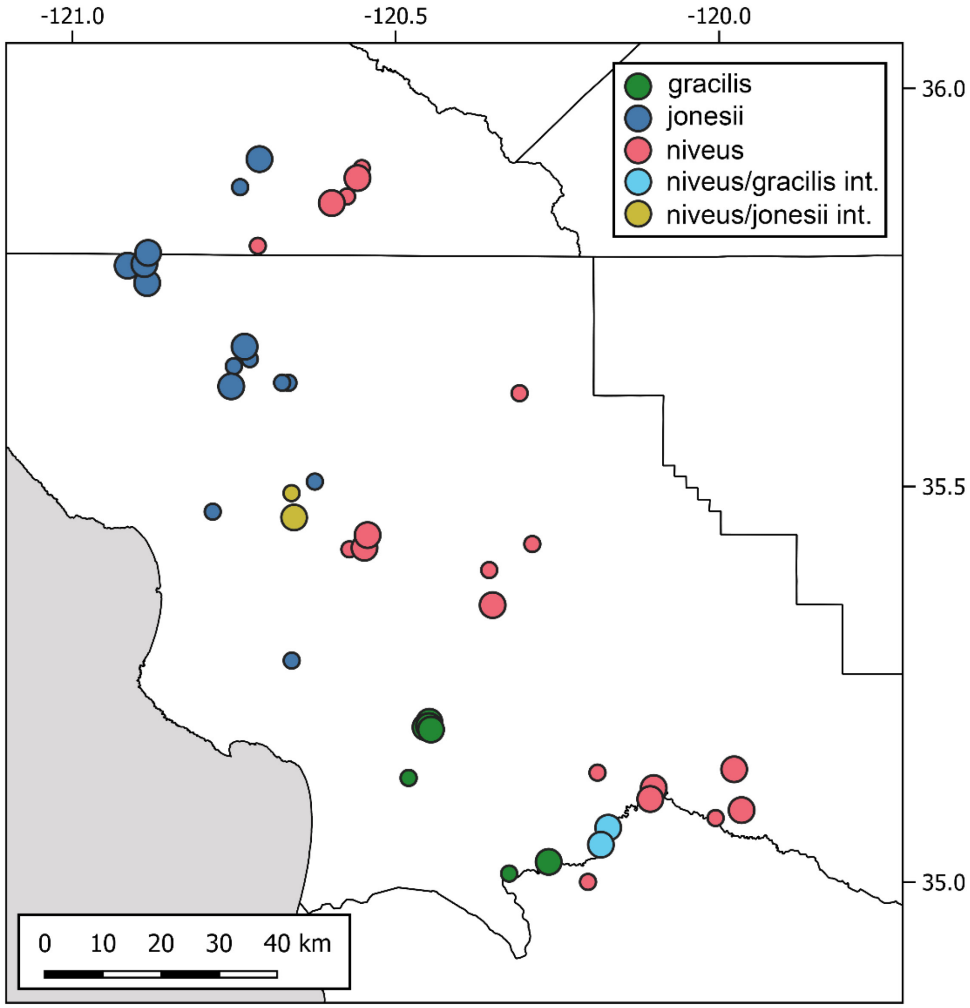

Figure 16. Map of the *Malacothamnus jonesii* clade with *M. jonesii* s.s., *M. gracilis*, *M. niveus*, and morphological and geographic intermediates of *M. niveus* with both *M. gracilis* and *M. jonesii*. Smaller circles indicate specimens measured in morphological analyses but not used in phylogenetic analyses. Larger circles indicate specimens used in phylogenetic analyses. Black lines indicate boundaries of California counties.

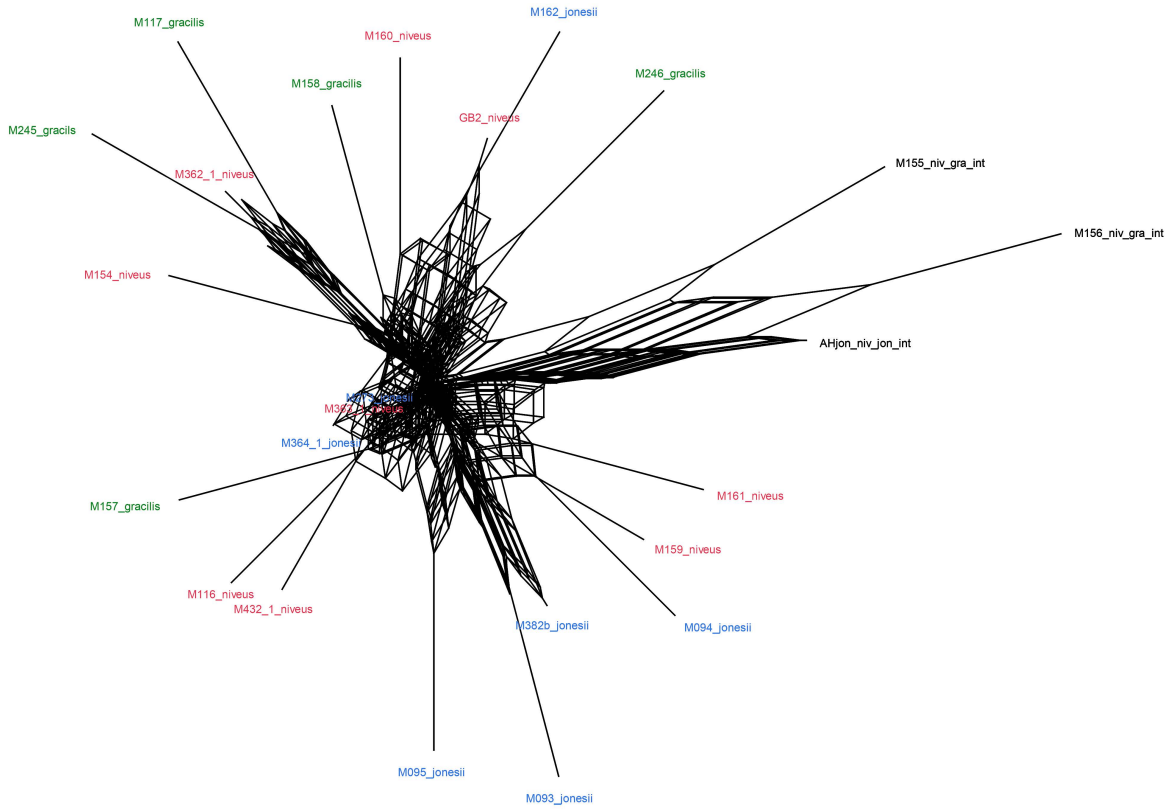

Figure 17. Neighbor net analysis of the *Malacothamnus jonesii* clade with *M. jonesii* s.s. (blue), *M. gracilis* (green), *M. niveus* (pinkish), and morphological and geographic intermediates of *M. niveus* with both *M. gracilis* and *M. jonesii* (black).

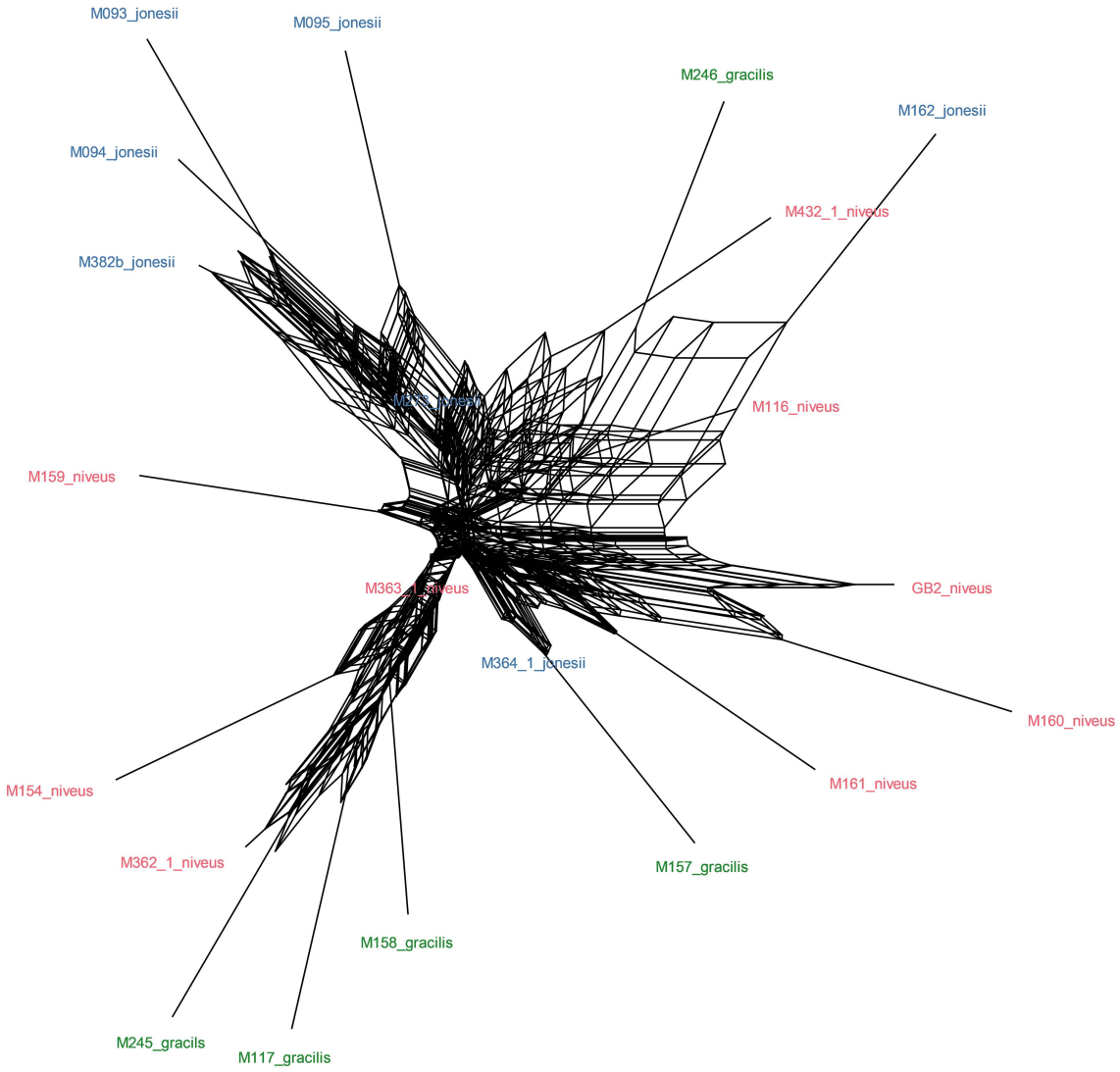

Figure 18. Neighbor net analysis of the *Malacothamnus jonesii* clade excluding morphological and geographic intermediates between species. *M. jonesii* s.s. (blue), *M. gracilis* (green), *M. niveus* (pinkish)

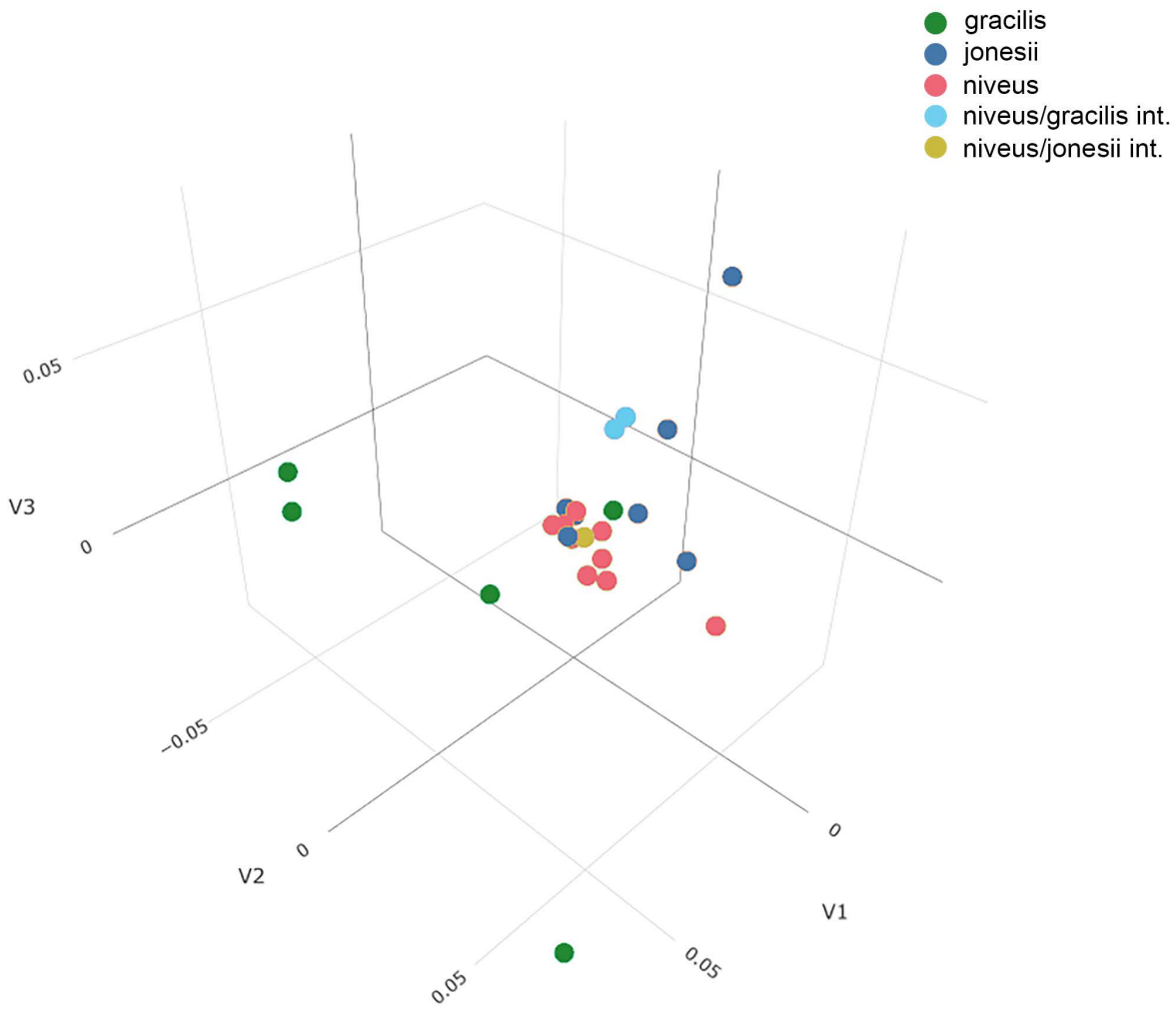

Figure 19. MDS of the *Malacothamnus jonesii* clade with *M. jonesii* s.s., *M. gracilis*, *M. niveus*, and morphological and geographic intermediates of *M. niveus* with both *M. gracilis* and *M. jonesii*.

5.4 *Malacothamnus davidsonii*

Malacothamnus davidsonii is known from two regions in California disjunct by ~330 km. Morphological analyses indicate plants from these two regions are significantly different; however, there is overlap in the PCA and 28% of specimens were misidentified in linear discriminant analysis. The combination of morphological similarity and geographic disjunction between northern and southern *M. davidsonii* indicates the possibility of convergent evolution or that they may be diverging from a relatively recent and morphologically similar common ancestor. See Volume 1 (Morse 2023) for more details on the morphological analyses.

In ML and Tetrad phylogenetic analyses, plants from Los Angeles County (the type locality) were monophyletic within the larger Southern Clade and plants from Monterey and San Luis Obispo counties were monophyletic within the larger Northern Clade, which indicates that they were not closely related to each other. Neighbor-net analysis and MDS were done on samples from both regions of *M. davidsonii* with sympatric taxa to test whether removing the restrictions of a bifurcating tree would yield different results.

Neighbor-net analysis of the northern and southern clades of *Malacothamnus davidsonii* with *M. abbottii* (sympatric with northern *M. davidsonii*) and *M. fasciculatus* var. *laxiflorus* from Los Angeles County (sympatric with southern *M. davidsonii*) shows each as monophyletic with no clear indication of gene flow between groups (Fig. 20). The network also indicates that each of the *M. davidsonii* groups is more closely related to the taxa they co-occur with than each other. MDS of the same groups (Fig. 21) follows the patterns seen in neighbor-net analysis.

Despite being very difficult to distinguish morphologically, as there is solid phylogenetic evidence separating the two clades of *M. davidsonii* and as their ranges are separated by over 300 km, I recognize the northern clade of what has been called *M. davidsonii* in the past from Monterey and San Luis Obispo counties as the new species *Malacothamnus discombobulatus*, which is described in Chapter 6 of this volume.

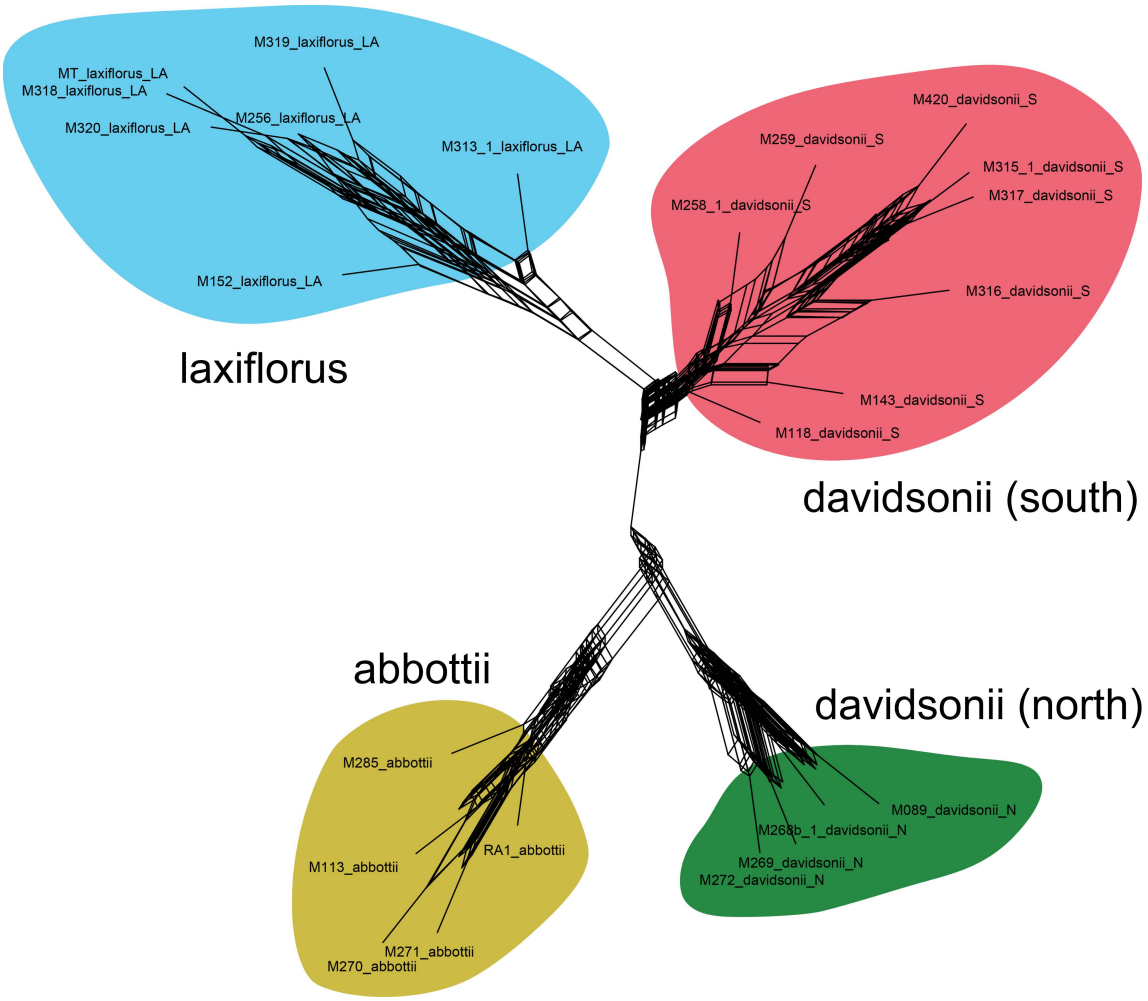

Figure 20. Neighbor-net analysis of the northern and southern regions of *Malacothamnus davidsonii* with sympatric taxa. *M. abbottii* is sympatric with northern *M. davidsonii* and *M. fasciculatus* var. *laxiflorus* from Los Angeles County is sympatric with southern *M. davidsonii*.

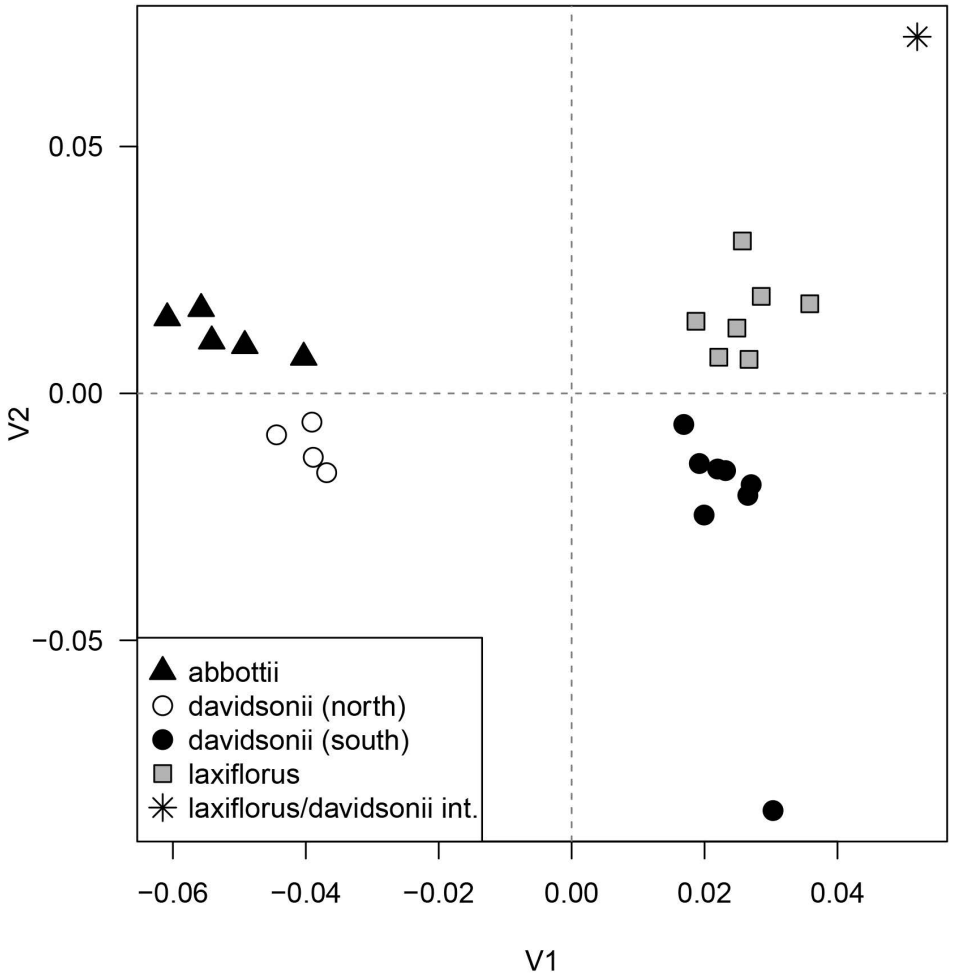

Figure 21. MDS of the northern and southern regions of *Malacothamnus davidsonii* with sympatric taxa. *M. abbottii* is sympatric with northern *M. davidsonii* and *M. fasciculatus* var. *laxiflorus* from Los Angeles County is sympatric with southern M. davidsonii. Also included is a morphologically intermediate plant of *M. davidsonii* (south) with *M. fasciculatus* var. *laxiflorus*.

5.5 *Malacothamnus densiflorus* sensu Bates

Malacothamnus densiflorus includes two named varieties, *M. d*. var. *densiflorus* and *M. d.* var. *viscidus*, that are morphologically distinct with no apparent intermediates. *Malacothamnus densiflorus* var. *viscidus* is primarily distinguished from the rest of the species based on trichome length and density, characters which hold up in a common garden. The range of *M. d.* var. *viscidus* is geographically adjacent to part of the southern range of *M. d*. var. *densiflorus* with a small amount of overlap between these ranges (Fig. 22). Morphological analysis also indicated a slight divergence between plants of *M. d*. var. *densiflorus* from the San Jacinto Mountains (type locality) and the rest of *M. d*. var. *densiflorus*. Plants of *M. d*. var. *densiflorus* from both the San Jacinto Mountains and the Santa Ana Mountains are geographically adjacent to populations of *M. fasciculatus* var. *laxiflorus* and slightly disjunct from the rest of *M. d*. var. *densiflorus*, which is mostly in San Diego County, CA. See Volume 1 (Morse 2023) for more details on the morphological analyses.

In ML and Tetrad analysis (Figs. 2 and 6), *Malacothamnus densiflorus* var. *viscidus* is generally monophyletic or mostly monophyletic. Placement of the clade relative to other taxa varies but it is usually within a larger clade that contains most of the rest of *M. densiflorus* and *M. enigmaticus*. Placement of *M. d.* var. *densiflorus* is variable (Figs. 2, 3, and 6). Specimens from both the Santa Ana Mountains and San Diego County are generally within a larger clade of *M. densiflorus* and *M. enigmaticus*, which are each mostly separated. Santa Ana Mountains plants generally cluster together, though only three plants were sampled. San Diego plants cluster less clearly with clades often including some specimens of the geographically adjacent *M. fasciculatus* var. *fasciculatus* and *M. enigmaticus*. Plants from the San Jacinto Mountains consistently form a clade within or sister to eastern plants of *M. fasciculatus* var. *laxiflorus*, which they are geographically adjacent to.

Neighbor-net analysis of just *Malacothamnus densiflorus* (Fig. 23) doesn't clearly differentiate any group, however, most plants of *M. d.* var. *viscidus* branch in one direction and most plants of var. *densiflorus* from the San Jacinto Mountain and Santa Ana Mountains branch in another. MDS of *M. densiflorus* (Fig. 24) is even less clear. MDS including just *M. d.* var. *densiflorus* and eastern *M. fasciculatus* var. *laxiflorus* (Fig. 25) shows the San Jacinto Mountains plants of *M. d.* var. *densiflorus* as intermediate between San Diego County plants and *M. fasciculatus* var. *laxiflorus*, which may indicate some hybridization in the past. Santa Ana Mountains plants are also intermediate or more aligned with *M. fasciculatus* var. *laxiflorus* in the plot.

Based on the total evidence, *Malacothamnus densiflorus* var. *viscidus* is morphologically distinct and mostly geographically distinct from the rest of *M. densiflorus* but it is unclear what the relationships between the varieties are. It is worth noting that the range of *M. d.* var. *viscidus* is similar to several other narrowly endemic taxa in the Otay Mountain region believed to have evolved in response to the region's unique soils. While *M. d.* var. *viscidus* could arguably be treated as a species, the fact that the main morphological difference between it

and the rest of the species may only be trichomes means it may be better to treat it as a variety unless more conclusive evidence can be provided for recognizing it as a species.

While *Malacothamnus densiflorus* var. *densiflorus* is not monophyletic and there appears to be some morphological divergence between regions, I believe gene flow with geographically adjacent species may be confounding the phylogenetic analyses. As *M. d.* var. *densiflorus* forms a morphologically cohesive group that can be easily distinguished from the rest of the genus, I believe it best to consider the divergences between regions minor. Whether the type locality of *M. densiflorus* should be considered hybrids with *M. fasciculatus* var. *laxiflorus* and whatever that would mean taxonomically is left to a future researcher. Samples from additional populations in the San Jacinto Mountains would be needed to explore the full amount of variation there relative to the rest of the species and ideally type specimens would be sampled and compared to the rest.

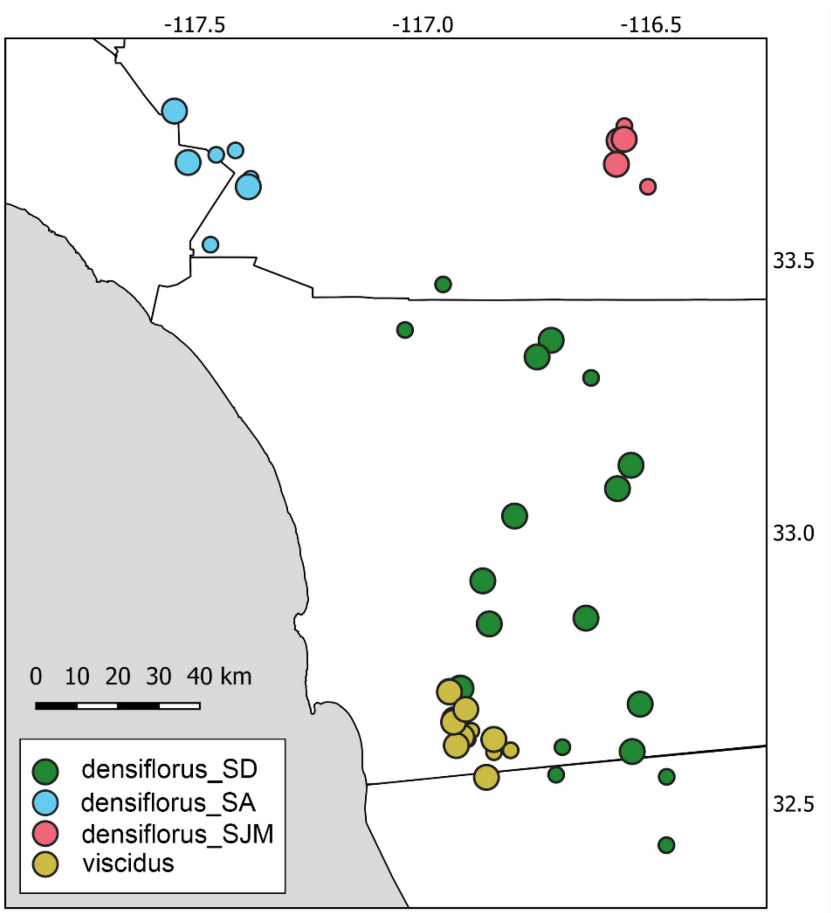

Figure 22. Map of *Malacothamnus densiflorus* var. *viscidus* and three geographic regions of *M. densiflorus* var. *densiflorus*. SA = Santa Ann Mountains. SD = San Diego County. SJM = San Jacinto Mountains (type locality). Smaller circles indicate specimens measured in morphological analyses but not used in phylogenetic analyses. Larger circles indicate specimens used in phylogenetic analyses. Black lines indicate boundaries of California counties.

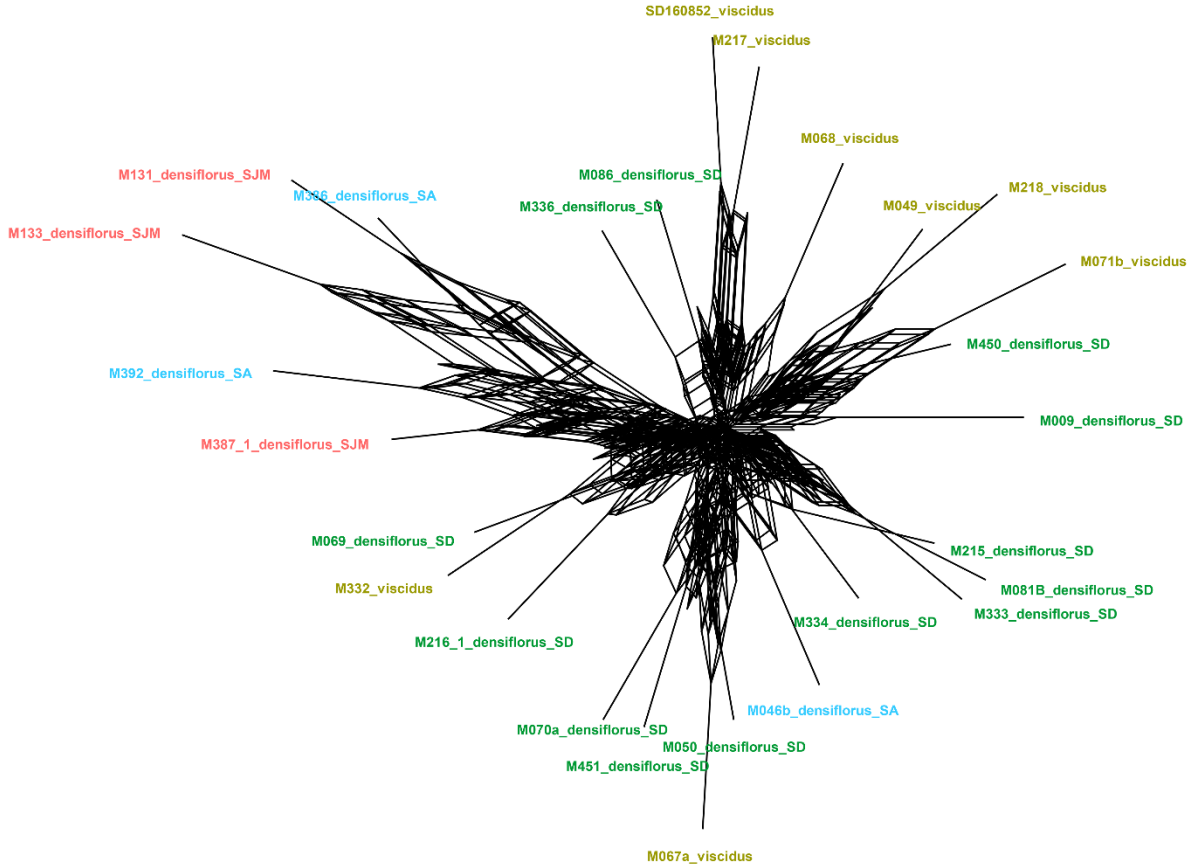

Figure 23. Neighbor-net analysis of *Malacothamnus densiflorus* var. *viscidus* (yellowish) and three geographic regions of *M. densiflorus* var. *densiflorus* (see below). Location abbreviations in suffixes of *M. densiflorus* are SA (Santa Ana Mountains = cyan), SD (San Diego County and adjacent Baja CA = green), and SJM (San Jacinto Mountains – the type locality = pinkish).

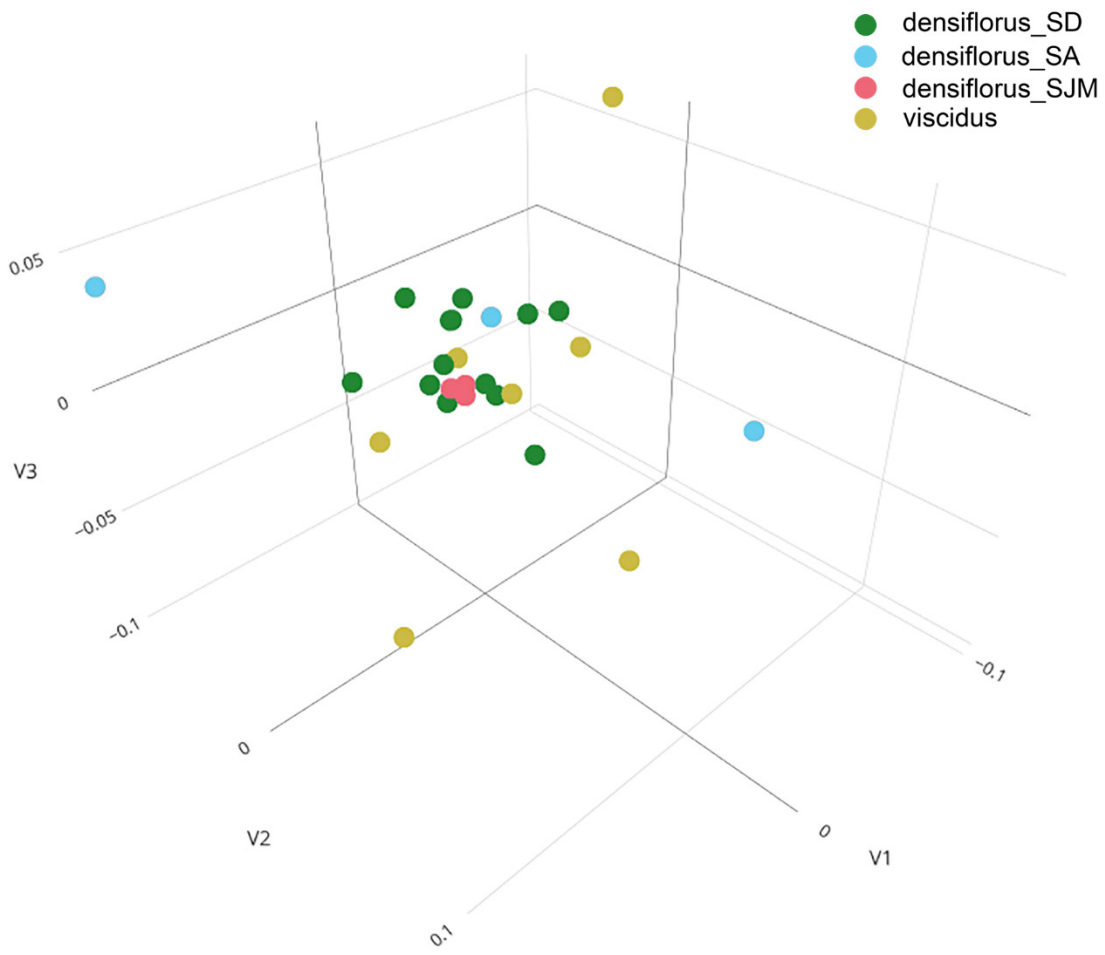

Figure 24. MDS of *Malacothamnus densiflorus* var. *viscidus* and three geographic regions of *M. densiflorus* var. *densiflorus*. SA = Santa Ann Mountains. SD = San Diego County. SJM = San Jacinto Mountains (type locality).

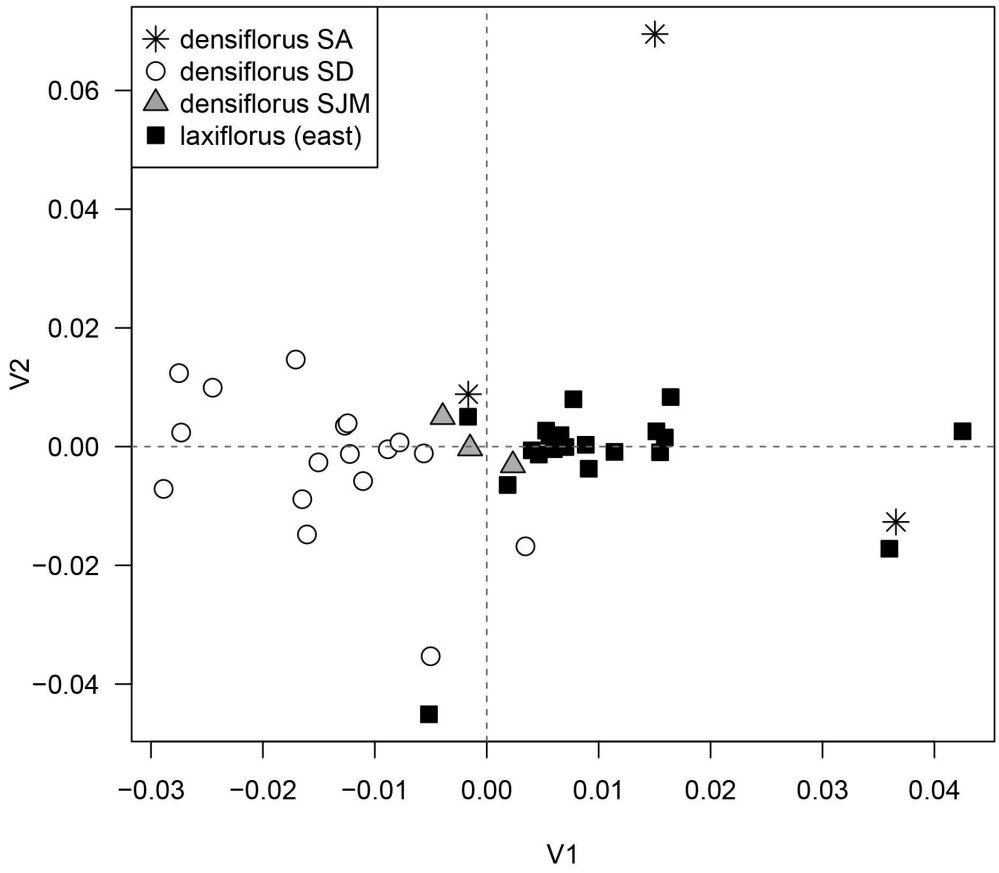

Figure 25. MDS of three regions of *Malacothamnus densiflorus* var. *densiflorus* with eastern *M. fasciculatus* var. *laxiflorus*. SA = Santa Ann Mountains. SD = San Diego County. SJM = San Jacinto Mountains (type locality).

5.6 *Malacothamnus fasciculatus* varieties

Kearney (1951) included five varieties of *Malacothamnus fasciculatus* (*catalinensis, fasciculatus, laxiflorus, nesioticus*, and *nuttallii*) in his treatment, which are mostly distinguishable in morphological analyses, though there are some intermediates (Morse 2023). There is some geographic isolation of *M. f.* var. *catalinensis*, *M. f.* var. *nesioticus*, and *M. f.* var. *nuttallii* from all other varieties but *M. f.* var. *fasciculatus* and *M. f.* var. *laxiflorus* appear to intergrade where their ranges meet. See Volume 1 for details on the morphological analyses.

5.6.1 *Malacothamnus fasciculatus* varieties ML analysis

In phylogenetic ML analyses of the full genus, each *Malacothamnus fasciculatus* variety has some level of support for distinctness; however, this is somewhat confounded by *M. fasciculatus* var. *laxiflorus*, which is polyphyletic (Figs. 2, 3, and 6). Eastern plants of *M. f.* var. *laxiflorus* are always monophyletic and sister to southern *M. davidsonii* or San Jacinto Mountains plants of *M. densiflorus* var. *densiflorus*. Western plants of *M. f.* var. *laxiflorus* are often polyphyletic and forming a larger clade that includes or is sister to *M. marrubioides*, generally includes a monophyletic *M. f.* var. *nesioticus*, and always includes a monophyletic *M. f.* var. *catalinensis*.

As the varieties of *Malacothamnus fasciculatus* do not form a single clade in ML and Tetrad analyses of the full genus but do pair up with geographically adjacent species, gene flow between geographically adjacent species could possibly be confounding relationships between the *M. fasciculatus* varieties in analyses of the full genus. To test whether the relationships between the varieties of *M. fasciculatus* differed without influence from adjacent species, I ran a ML analysis of just the *M. fasciculatus* varieties (Fig. 26).

In this analysis, *M. f.* var. *nesioticus* is a moderately supported sister to *M. f.* var. *nuttallii* rather than within a clade of western *M. f.* var. *laxiflorus*. Eastern *M. f.* var. *laxiflorus* remains a well-supported clade and separate from western *M. f.* var. *laxiflorus.* Western *M. f.* var. *laxiflorus* is split into three clades that mostly include the samples of the three western *M. f.* var. *laxiflorus* clades in Figure 3. Clade 1 is sister to the Santa Catalina Island endemic *M. f.* var. *catalinensis* with all samples on the mainland geographically clustered north of *M. f.* var. *catalinensis.* Clade 2 is sister to *M. f.* var. *fasciculatus* with most samples geographically adjacent to *M. f. var. fasciculatus* and eastern *M. f.* var. *laxiflorus*, though a region of *M. densiflorus* in the Santa Ana Mountains partially separates eastern *M. f.* var. *laxiflorus* from Clade 2. One sample from Clade 2 is found in the geographic range of *M. f.* var. *nuttallii* and was presumably a poorly chosen planting for a restoration project. Clade 3 forms a geographic cluster adjacent to *M. marrubioides* with three of the four samples in the clade being sister to *M. marrubioides* in Figure 3.

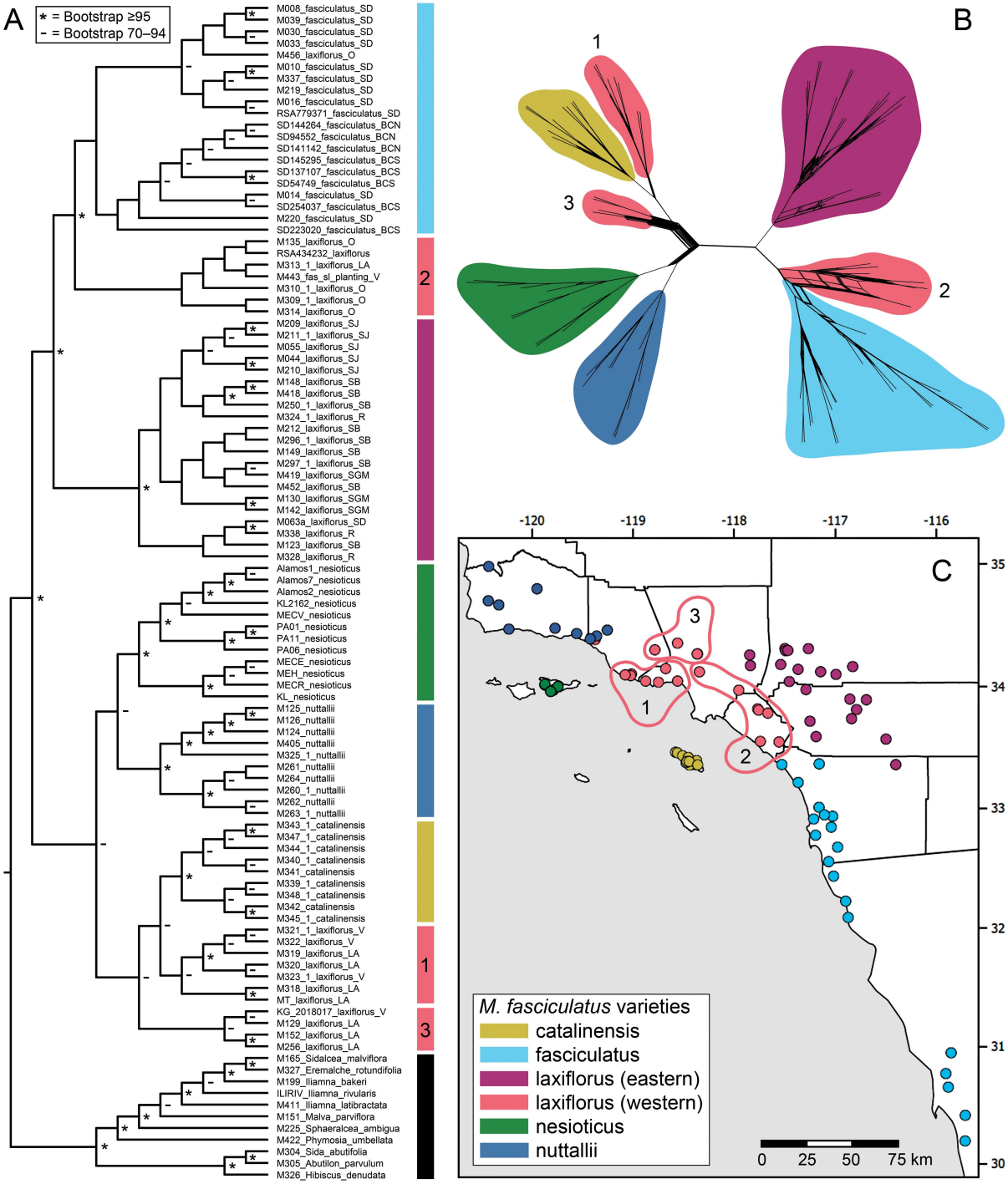

Figure 26. A) Consensus ML tree of the varieties of *Malacothamnus fasciculatus*. B) Phylogenetic network showing bootstrap conflict from A excluding outgroups. C) Map of samples included. Western *M. f.* var. *laxiflorus* forms three clades, the numbering of which corresponds to the numbering in Figure 3. In C, one barely visible point of western *M. f.* var. *laxiflorus* under points of *M. f.* var. *nuttallii* is an apparent restoration planting possibly sourced from the range of western *M. f.* var. *laxiflorus* Clade 2. The large gap in the range of *M. f.* var. *fasciculatus* shown includes both *M. foliosus* and *M. fasciculatus/foliosus* intermediates. *Malacothamnus marrubioides*, which may have or had gene flow with western *M. f.* var. *laxiflorus* Clade 3, occurs near the number 3. The type locality of *M. f.* var. *laxiflorus* is in the geographic range of Clade 1.

5.6.2 *Malacothamnus fasciculatus* varieties neighbor-net analyses

Because ML analyses indicate western populations of *Malacothamnus fasciculatus* var. *laxiflorus* may serve as a geographic hub connecting the other varieties of *M. fasciculatus* together, I ran neighbor-net analyses on several combinations of the *M. fasciculatus* varieties to try to further clarify relationships among them (Fig. 27).

Neighbor-net analysis of just *Malacothamnus fasciculatus* var. *laxiflorus* is consistent with the ML analyses in that samples in the eastern *M. f.* var. *laxiflorus* clade do not cluster with the western samples (Fig. 27A). Western samples form a single cluster with subclusters somewhat corresponding to the clades in Figure 26.

When *Malacothamnus fasciculatus* var. *laxiflorus* is separately analyzed with each *M. f.* var *nesioticus* and *M. f.* var. *nuttallii*, western *M. f.* var. *laxiflorus* forms two clusters, one including Clade 2 and the other including both Clades 1 and 3 (Fig. 27B & C). Both *M. f.* var *nesioticus* and *M. f.* var. *nuttallii* form distinct clusters in these analyses, though one sample of *M. f.* var. *laxiflorus* (RSA434232) is part of the *M. f.* var *nesioticus* cluster.

When *Malacothamnus fasciculatus* var. *laxiflorus* is separately analyzed with each *M. f.* var. *catalinensis* and *M. f.* var *fasciculatus*, eastern *M. f.* var. *laxiflorus* remains mostly distinct but the other varieties are not distinct from western *M. f.* var. *laxiflorus* (Fig. 27D & E).

Note in Figure 26 that *Malacothamnus fasciculatus* var. *fasciculatus* forms two clades. One consists mostly of plants from San Diego County that likely have geneflow with adjacent *M. f.* var. *laxiflorus* and/or *M. densiflorus*. The other consists mostly of Baja California plants that likely have geneflow with *M. foliosus*. Reducing the *M. f.* var. *fasciculatus* samples to just those from San Diego County possibly also reduces conflicting signal from possible geneflow with *M. foliosus* and improves clustering (Fig. 27F). In this analysis, *M. f.* var. *laxiflorus* Clade 1 forms a separate cluster. Clade 2 forms a cluster with San Diego County *M. f.* var. *fasciculatus*, which they are geographically adjacent to. Clade 3 appears possibly intermediate between Clade 1 and 2.

Three samples that occurred in multiple clades in ML analyses are identified separately in Figure 27. Specimen M063a is from the northern end of the range of *Malacothamnus enigmaticus* and is morphologically most similar to nearby *M. fasciculatus* var. *laxiflorus*. In phylogenetic ML analyses of the full genus, it falls out in clades dominated by *M. enigmaticus*. The combination of geographic location, morphology, and ML analyses indicate it may be intermediate between *M. enigmaticus* and *M. f.* var. *laxiflorus*. In Figure 27, M063a is on the edge of the eastern *M. f.* var. *laxiflorus* in four networks and in other clusters in two networks.

Specimen RSA434232 is inconsistent in the networks of Figure 27. This specimen was collected in a somewhat geographically intermediate area between the *Malacothamnus fasciculatus* var. *laxiflorus* clades and can be seen as the northernmost specimen in Clade 2 in Figure 26. This indicates that it may be intermediate between ML clades, which could cause it

to change output positions in different analyses. Possible lower quality of DNA due to the age of this specimen may also contribute to its inconsistency in analyses. This is the only sample included from an herbarium specimen over 100 years old.

Specimen KG_20170117 was collected from a geographically intermediate area between *Malacothamnus fasciculatus* var. *laxiflorus* Clades 1 and 3. In ML analysis of the full genus and neighbor-net analyses of just the *M. fasciculatus* varieties, it comes out in Clade 1 (Fig. 3, Fig. 27). However, in ML analysis of just the *M. fasciculatus* varieties (Fig. 26), it comes out in Clade 3.

5.6.3 *Malacothamnus fasciculatus* varieties MDS analyses

MDS of all samples from all *Malacothamnus fasciculatus* varieties (Fig. 28A) is dominated by the large spread of *M. f.* var. *fasciculatus* samples including several outliers from Baja California, which overly compresses most samples into the center of the plot and masks signal distinguishing some other varieties. This is similar to the problem encountered when including Baja California samples in Figure 27E. Removing the Baja California samples as done in Figure 27F yields a clustering where both *M. f.* var. *nuttallii* and eastern plants of *M. f.* var. *laxiflorus* form distinct clusters separate from the rest (Fig. 28B). Removing *M. f.* var. *nuttallii* and eastern plants of *M. f.* var. *laxiflorus* from the remaining samples differentiates *M. f.* var. *nesioticus* from the rest (Fig. 28C). Further removing *M. f.* var. *nesioticus* from the remaining samples shows clustering but without clear separation of the clusters (Fig. 28D). If the results from Figure 28D are plotted with western *M. f.* var. *laxiflorus* classified by the three clades from Figure 26, *M. f.* var. *fasciculatus* and western *M. f.* var. *laxiflorus* Clade 2 are together differentiated from the rest, which is consistent with both the ML and neighbor-net analyses of the *M. fasciculatus* varieties (Fig. 28E).

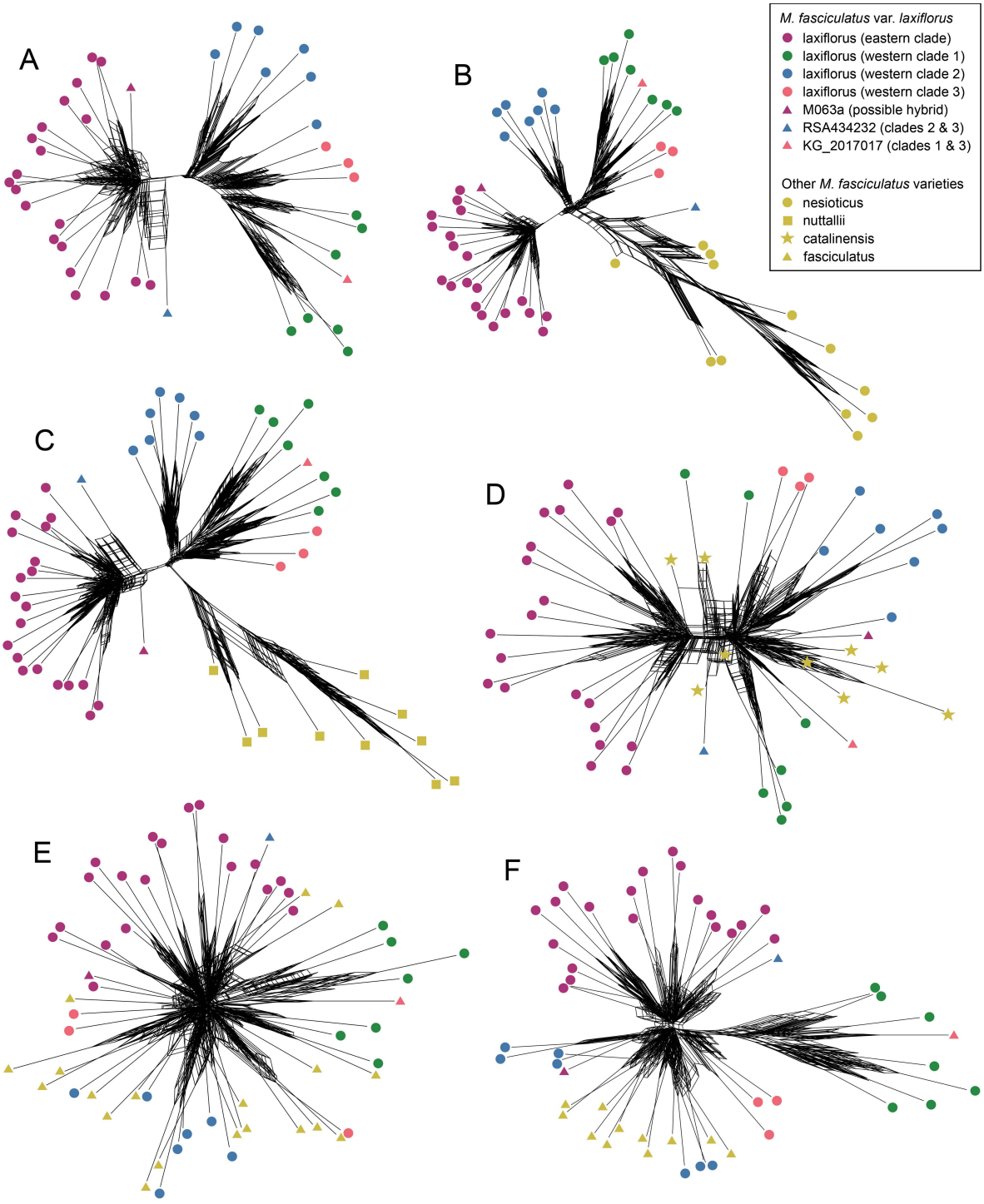

Figure 27. Neighbor-net analyses of *Malacothamnus fasciculatus* var. *laxiflorus* with the other varieties of *M. fasciculatus*. *Malacothamnus fasciculatus* var. *laxiflorus* is classified using its four clades from ML analyses. The type locality of *M. f.* var. *laxiflorus* is in the geographic range of Clade 1. Three *M. f.* var. *laxiflorus* samples that are inconsistent in which clade they are placed in ML analyses are each identified by their own symbol. A) Analysis including only *M. f.* var. *laxiflorus*. B) *M. f.* var. *laxiflorus* with *M. f.* var. *nesioticus*. C) *M. f.* var. *laxiflorus* with *M. f.* var. *nuttallii*. D) *M. f.* var. *laxiflorus* with *M. f.* var. *catalinensis*. E) *M. f.* var. *laxiflorus* with all *M. f.* var. *fasciculatus*. F) *M. f.* var. *laxiflorus* with San Diego County *M. f.* var. *fasciculatus*.

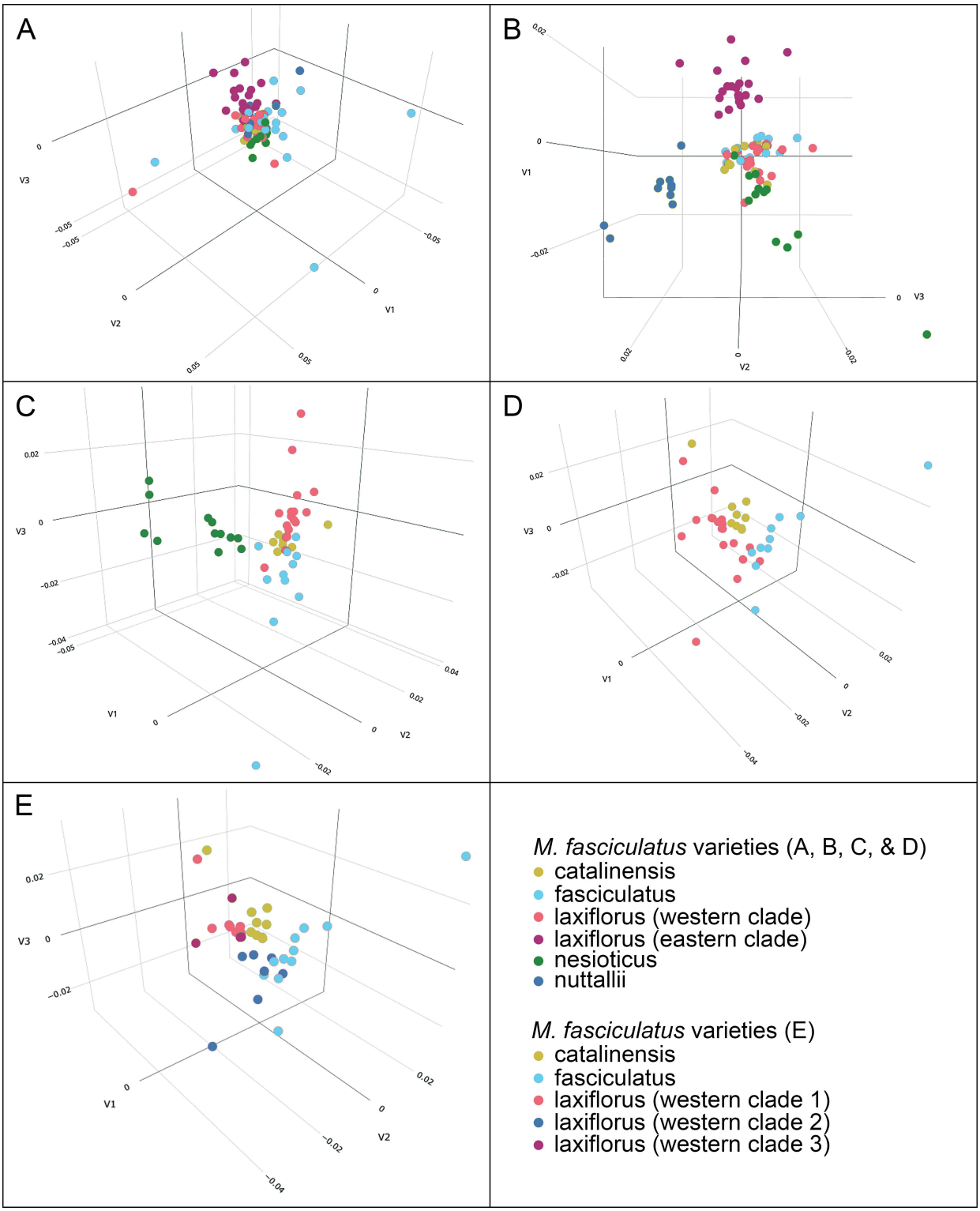

Figure 28. Sequential MDS of the varieties of *Malacothamnus fasciculatus*. A) All samples included. B) Same dataset as A but with samples of *M. f.* var. *fasciculatus* from Baja California excluded. C) Same dataset as B but with further exclusion of eastern *M. f.* var. *laxiflorus* and *M. f.* var. *nuttallii*. D) Same dataset as C but with further exclusion of *M. f.* var. *nesioticus*. E) Same dataset as D except western *M. f.* var. *laxiflorus* clades labelled separately.

5.6.4 *Malacothamnus fasciculatus* varieties conclusions

Malacothamnus fasciculatus var. *nuttallii* is the only variety that is not included in the larger southern California clade in analyses of the full genus and has no clear connection to *M. f.* var. *laxiflorus* in analyses of just the *M. fasciculatus* varieties. *Malacothamnus fasciculatus* var. *nuttallii* is also generally easily distinguished morphologically from the rest of *M. fasciculatus* and has a peak bloom time roughly one month later than the rest of *M. fasciculatus* at similar elevations. As *M. f.* var. *nuttallii* has several lines of evidence differentiating it from the rest of *M. fasciculatus*, I recognize it at the species rank as *M. nuttallii*.

Due to both morphological and phylogenetic ambiguities between the remaining varieties of *Malacothamnus fasciculatus*, particularly associated with the western region of *M. f.* var. *laxiflorus*, it seems best to retain these varieties until a future researcher can clarify the relationships between them. This allows for ambiguous specimens to be classified to the species rank. Both *M. f.* var. *catalinensis* and *M. f.* var. *nesioticus* are monophyletic island endemics, which suggests they are independently evolving lineages, though each are often within a larger clade of western *M. f.* var. *laxiflorus* in phylogenetic analyses. Eastern *M. f.* var. *laxiflorus* appears to be distinct from the rest of *M. fasciculatus* in phylogenetic analyses, however, it is morphologically ambiguous from western plants of *M. f.* var. *laxiflorus* and there are some geographic gaps in the phylogenetic sampling that, if sampled, could possibly change the outcome of the analyses. *Malacothamnus fasciculatus* var. *fasciculatus* may be mostly phylogenetically distinct from the rest but its relationship to *M. f.* var. *laxiflorus* needs further investigation. Future phylogenetic analyses need to include a type specimen of the presumed extinct *M. parishii*, which I treat as an extreme form of *M. f.* var. *laxiflorus.* If *M. parishii* is not distinct from the eastern clade of *M. f.* var. *laxiflorus* but the eastern clade is distinct from the rest of *M. fasciculatus*, *M. parishii* would be the name to assign to that clade.

5.7 *Malacothamnus foliosus* sensu Bates

Malacothamnus foliosus sensu Bates includes both *M. foliosus* and *M. paniculatus*. Morphological analyses of *M. foliosus* sensu Bates indicated that it is perhaps reasonably treated as two morphological groups: a more coastal group containing no type specimens and a more inland group containing the types of both *M. foliosus* and *M. paniculatus* (Fig. 29). This is confounded by all specimens in the inland group excluding the *M. foliosus* types appearing to possibly be intermediate between the *M. foliosus* types and the coastal group. Both the *M. foliosus* and *M. paniculatus* type specimens are outliers in the morphological analyses. If *M. foliosus* sensu Bates were considered a single highly variable morphological group, the *M. foliosus* types would represent one extreme of the morphological spectrum and the coastal group would represent the opposite extreme. The *M. paniculatus* type is somewhat intermediate between the *M. foliosus* types and the coastal group but an outlier in some measurements relative to all other intermediate specimens. See Volume 1 (Morse 2023) for more details on the morphological analyses.

Phylogenetic analysis was somewhat limited in that no specimens sampled represent the morphological extremes seen in the types of *Malacothamnus foliosus* and *M. paniculatus*. ML analysis (Figs. 2 and 6) generally clustered the inland and coastal *M. foliosus* groups into a single clade but the inland and coastal groups themselves never sorted into distinct clades. Neighbor-net analyses (Fig. 30) and MDS (Fig. 31) also failed to show any differentiation between these groups.

Recognizing the coastal group as a variety of *Malacothamnus foliosus* may be justifiable if one concentrates on the morphological and geographic evidence. However, as the morphological extremes seen in the type specimens of both *M. foliosus* and *M. paniculatus* are only represented by the type specimens themselves, which are not included in the phylogenetic analyses, I think it best to include both the coastal and inland specimens together under *M. foliosus* without recognizing varieties until a future researcher can clarify the relationships between the type specimens and these morphological groups.

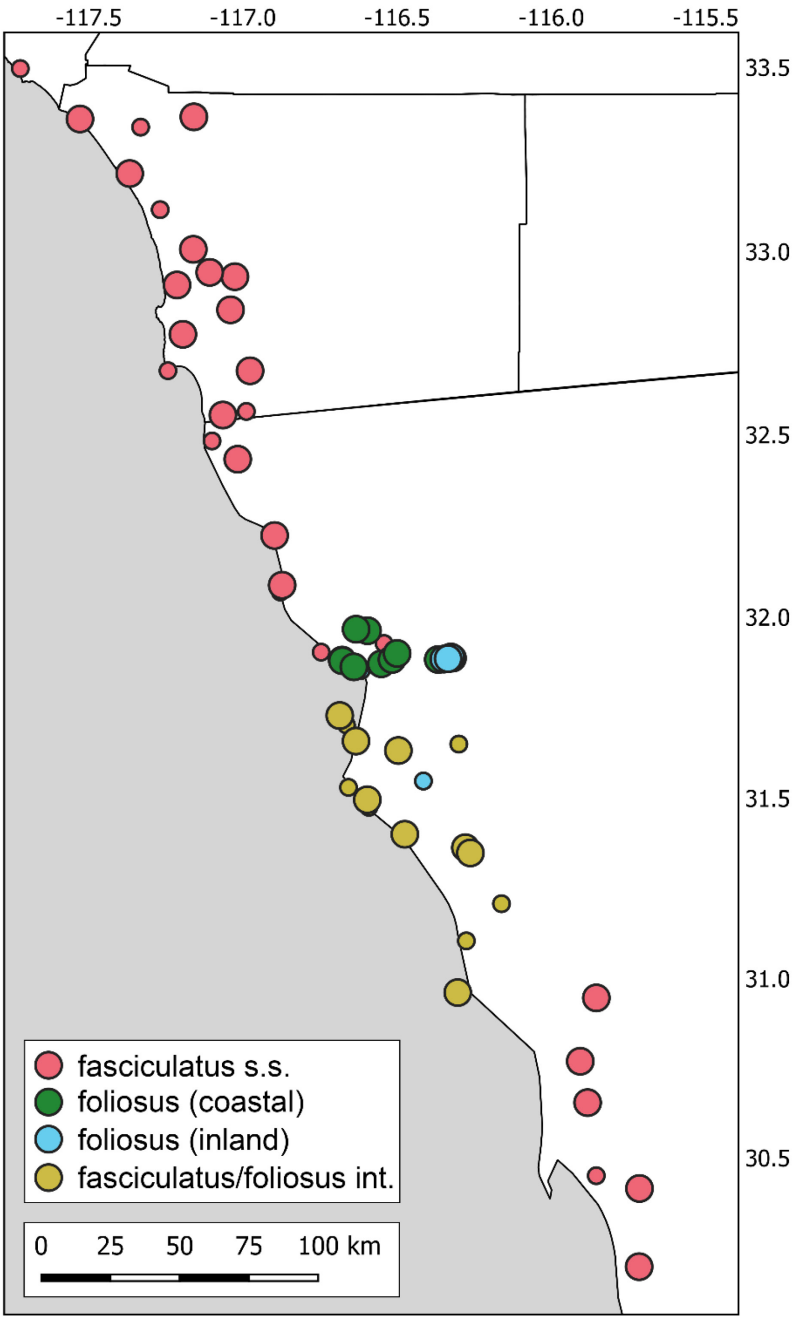

Figure 29. Map of *Malacothamnus fasciculatus* s.s., *M. foliosus* (coastal and inland forms), and *fasciculatus/foliosus* morphological intermediates. Smaller circles indicate specimens measured in morphological analyses but not used in phylogenetic analyses. The small blue circle is the purported type locality of *M. foliosus*. Larger circles indicate specimens used in phylogenetic analyses. Black lines indicate boundaries of California counties.

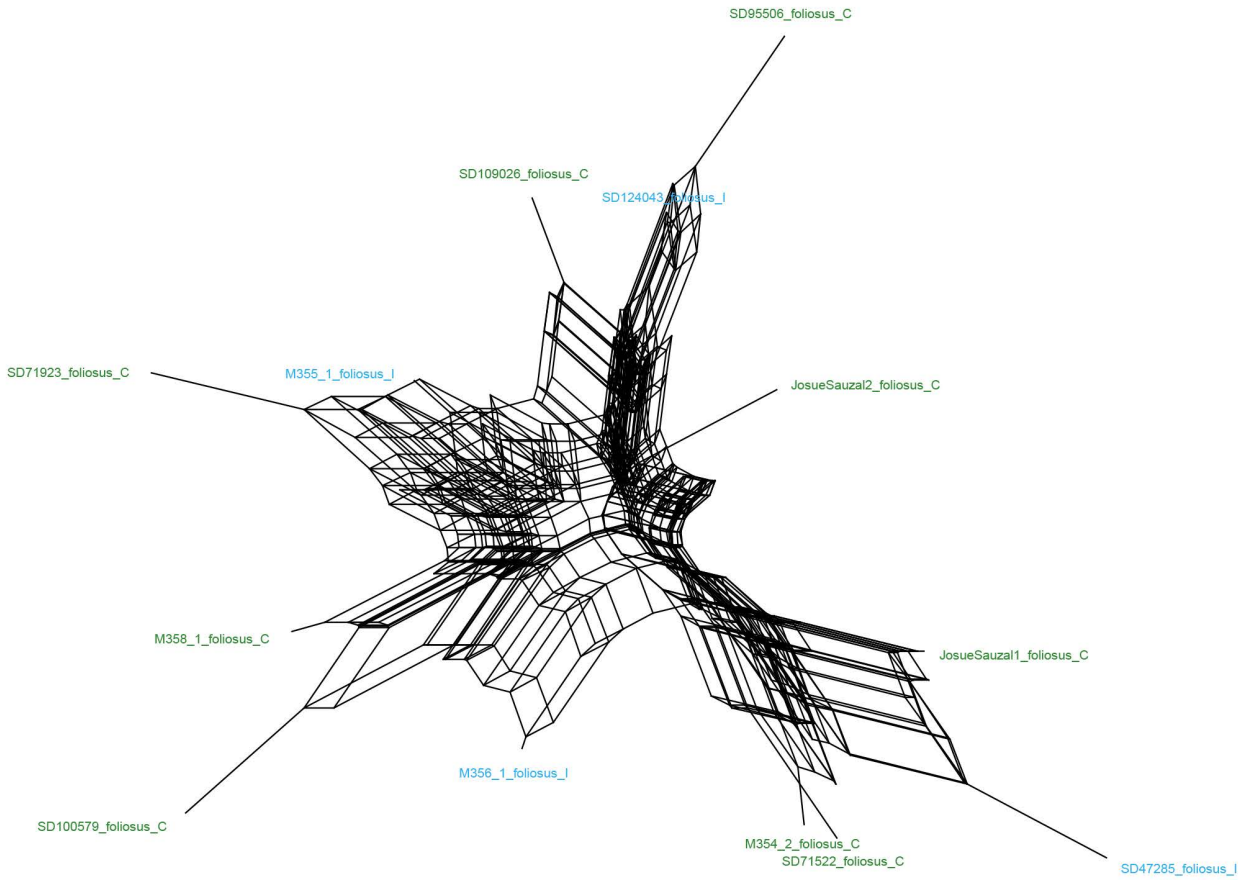

Figure 30. Neighbor-net analysis of *Malacothamnus foliosus* comparing the coastal (green) and inland (blue) morphological groups.

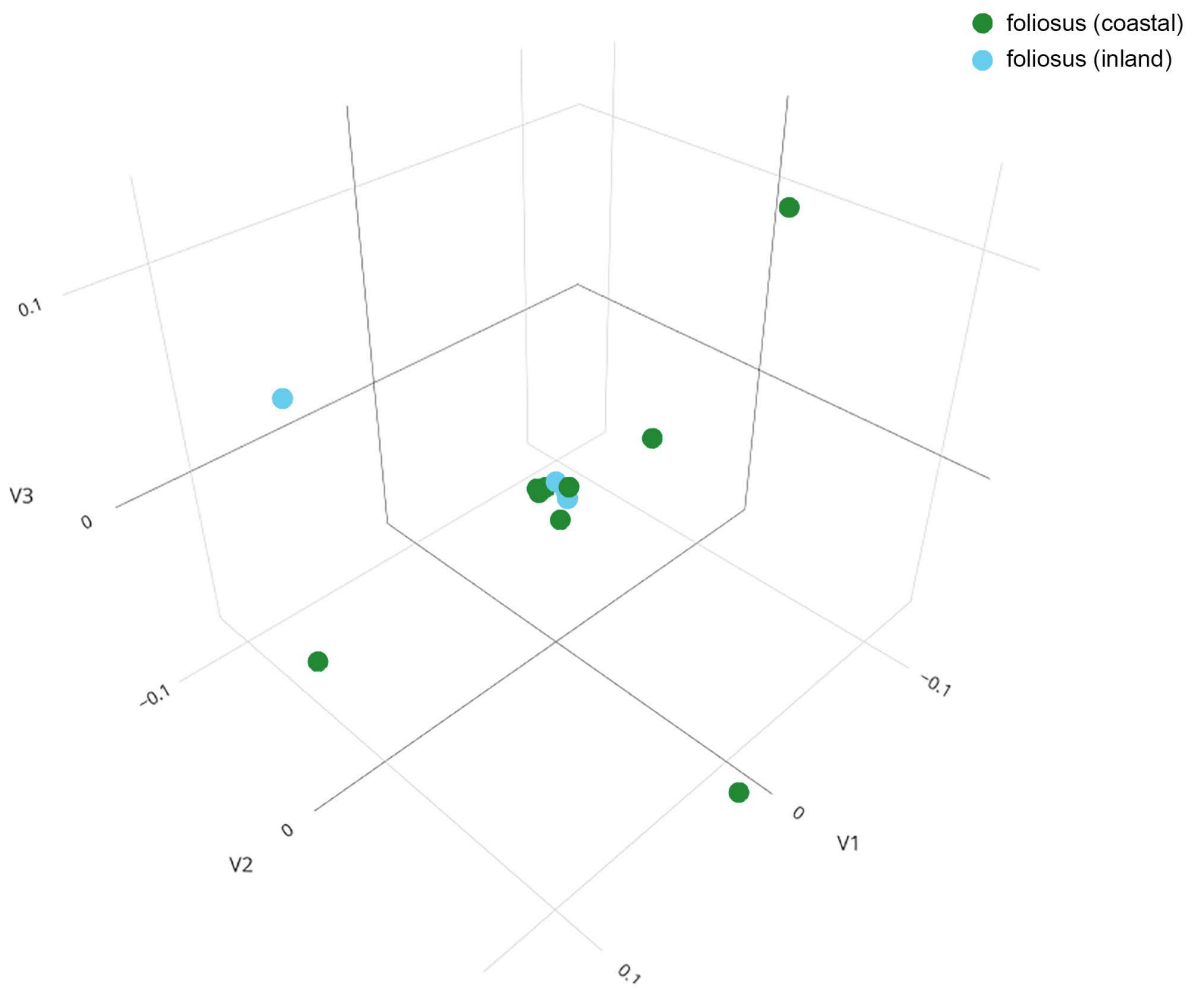

Figure 31. MDS of *Malacothamnus foliosus* comparing the coastal and inland morphological groups.

5.8 *Malacothamnus fasciculatus/foliosus* intermediates

In Volume 1 (Morse 2023), I proposed a morphological grouping called "*M. fasciculatus*/*foliosus* intermediates", which occurs geographically between *M. foliosus* and the southernmost populations of *M. fasciculatus* s.s (Fig. 29). Morphologically, this group is most similar to *M. fasciculatus* s.s. and has some morphological characters intermediate between *M. fasciculatus* s.s. and *M. foliosus*, however, characters are inconsistent between specimens. The *M. fasciculatus*/*foliosus* intermediates could be the result of hybridization between *M. fasciculatus* s.s. and *M. foliosus*. An alternate hypothesis would be that the apparent intermediates are an undescribed taxon. See Volume 1 for more details on the morphological analyses.

In phylogenetic ML analyses, the *M. fasciculatus*/*foliosus* intermediates are generally within a larger clade of both *M. fasciculatus* s.s. and *M. foliosus*, with *M. fasciculatus* s.s. sometimes split between this clade and one with *M. densiflorus* (Fig. 2). This provides evidence that at least some of the *M. fasciculatus*/*foliosus* intermediates are likely truly intermediates rather than a less closely related undescribed taxon. In neighbor-net analyses (Fig. 32), the intermediates are not distinguished from *M. fasciculatus* s.s. and *M. foliosus,* and MDS (Fig. 33) only offers vague hints of a pattern. Based on the apparent phylogenetic intermediacy of the *M. fasciculatus*/*foliosus* intermediates in ML analyses and that they are most morphologically similar to *M. fasciculatus* s.s., it may be best to include those least similar to *M. foliosus* within *M. fasciculatus* s.s. and consider those most similar to *M. foliosus* as likely hybrids. Population-level sampling is needed to more clearly determine if all apparent intermediates are actually intermediate and to what degree.

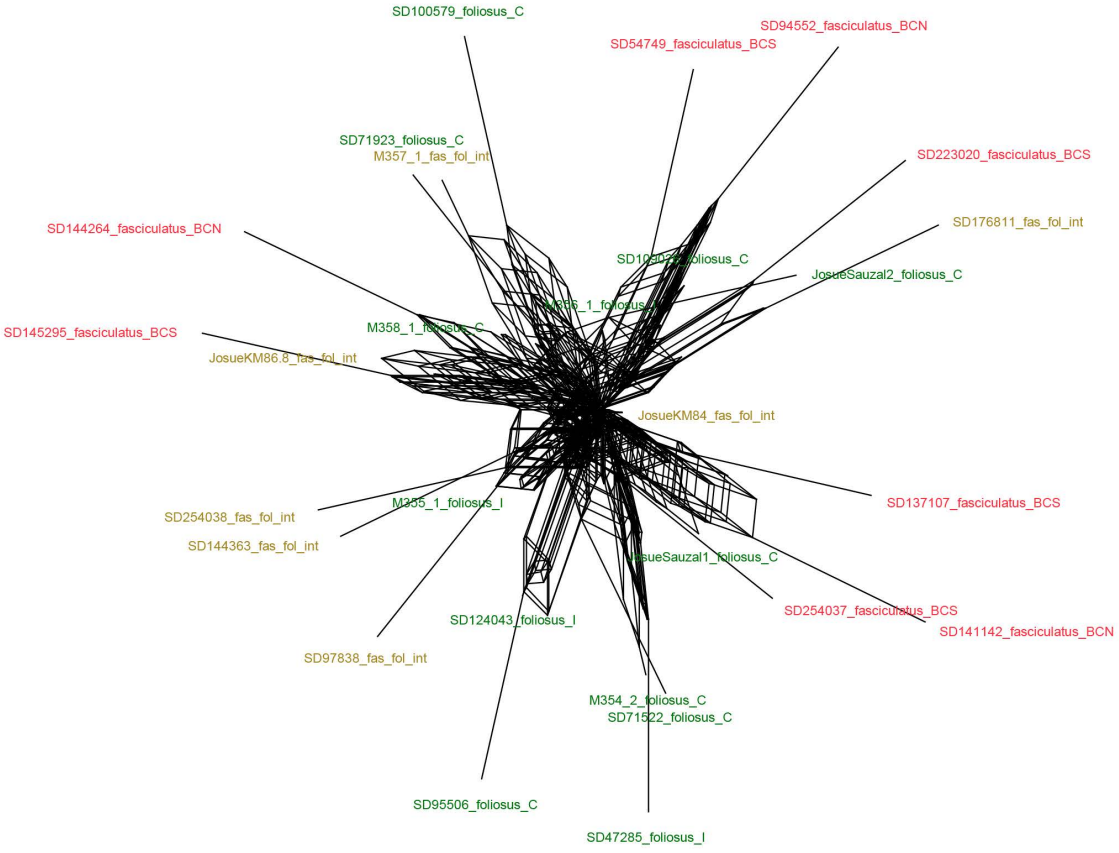

Figure 32. Neighbor-net analysis of Baja California samples of *Malacothamnus fasciculatus* s.s. (pinkish), *M. foliosus* s.l. (green), and the *fasciculatus*/*foliosus* intermediates morphological group (yellowish).

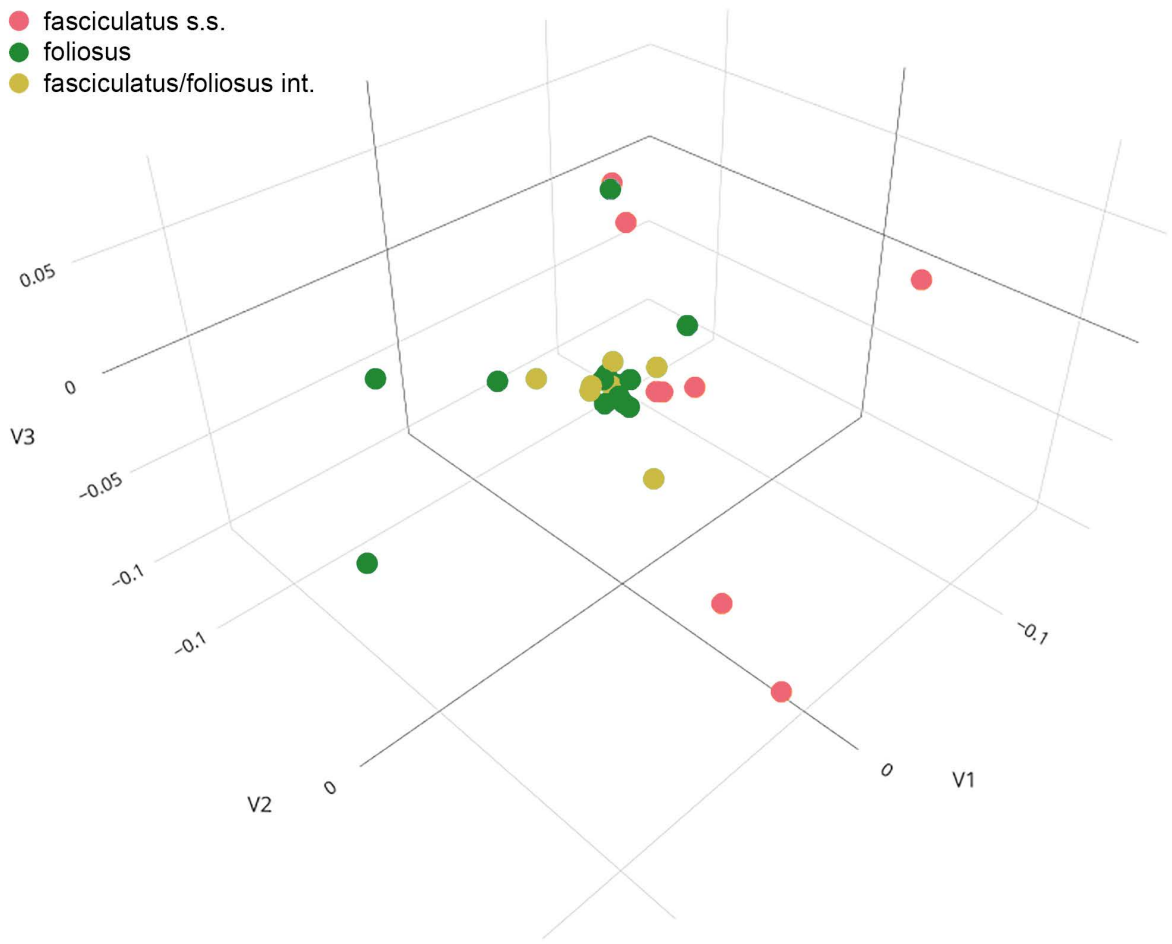

Figure 33. MDS of Baja California samples of *Malacothamnus fasciculatus* s.s., *M. foliosus* s.l., and the *fasciculatus/foliosus* intermediates morphological group.

5.9 "Baja East"

In Volume 1 (Morse 2023), I proposed a morphological grouping called "Baja East", which are plants from Baja California that are similar to *Malacothamnus enigmaticus* and have a geographic range that could be interpreted as a southward extension of the range of *M. enigmaticus*. Morphological analysis indicated that "Baja East" was most similar to *M. enigmaticus*, and that "Baja East" could possibly be included within *M. enigmaticus* or be an undescribed taxon. See Volume 1 for more details on the morphological analyses.

In phylogenetic analyses, "Baja East" is never monophyletic, and specimens are included in clades of all species from Baja California plus *M. enigmaticus*.

The “Baja East” group is very poorly sampled having only four specimens from the northern end of its range close to *M. enigmaticus* and a further two specimens from a single collection near the presumed southern end of its range 225 km to the south. iNaturalist observations indicate similar plants occur between the southern and northern ends of the “Baja East” range (iNaturalist 2022). A somewhat poorly documented iNaturalist observation also indicates the possibility of plants occurring geographically in-between *M. enigmaticus* and “Baja East”. The collective pattern of both specimens and iNaturalist observations indicates that *M. enigmaticus* and “Baja East” may form a continuous distribution that is currently well documented only at its most northern end. Further examination of specimens indicates that while “Baja East” specimens include some measurement extremes relative to *M. enigmaticus*, they cannot be clearly distinguished from *M. enigmaticus*. Based on the currently available evidence, I include “Baja East” within *M. enigmaticus* acknowledging that further evidence could justify some or all of “Baja East” being recognized as a distinct species or as a variety of *M. enigmaticus*.

5.10 “Baja East”/fasciculatus intermediates

In Volume 1 (Morse 2023), I proposed “Baja East”/fasciculatus intermediates as a geographically cohesive, but morphologically variable group found where the southern ranges of “Baja East” and *Malacothamnus fasciculatus* come together. This study suggested that they may represent hybrids or morphological intermediates of these groups. Only two specimens of “Baja East”/fasciculatus intermediates were included in phylogenetic analyses, which were inconsistent in their placement in ML analyses. Further sampling is needed to clarify how specimens in this group should be classified. For now, I consider them to be intermediate between *M. fasciculatus*, *M. enigmaticus*, and/or *M. foliosus*.

6 NEW SPECIES

The unified species concept (de Queiroz 2007) defines species based on the common element of previous species concepts, that species are separately evolving metapopulation lineages. This concept considers additional criteria used to define species in previous concepts as lines of evidence for this definition, recognizing that not all lineages will meet all these criteria or meet these criteria in the same chronological order. Three previously unnamed morphological and geographic groups assessed in this study clearly fit the definition of a species under this concept. The plants from Shasta County ("Shasta") and the plants from Vandenberg Space Force Base ("Vandenberg") are each morphologically distinct, geographically isolated from similar taxa, and monophyletic in phylogenetic analyses excluding hybrids. The geographically disjunct northern populations of *Malacothamnus davidsonii*, while very similar morphologically to plants from the southern type locality, form a distinct clade that is not closely related to the southern populations in phylogenetic analyses. I describe these three groups as new species.

6.1 *Malacothamnus eastwoodiae*

Plants from Vandenberg Space Force Base appear to have been suspected as an undescribed species since first vouchered in 1975 as most specimens are only identified to genus. In the 1976 flora of the Santa Barbara region, the author called it an "unknown taxon" (Smith 1976). In a 1985 environmental impact statement it was noted as an unnamed new species restricted to "an unusual form of maritime chaparral called Burton Mesa Chaparral" (Howald et al. 1985). In the most recent edition of the flora of the Santa Barbara region, it was treated as a scarce form of *M. fremontii* (Smith 1998).

The plants from Vandenberg are clearly distinct in morphological analyses. They were prominently separated from the rest of *M. fremontii* sensu Bates in a PCA (Volume 1 Fig. 33) primarily based on length of glandular trichomes. In an LDA of the full genus (Volume 1 Table 18), the Vandenberg plants were accurately predicted 100% of the time. In a morphological distance matrix of the full genus (Volume 1 Fig. 44), the Vandenberg specimens are most similar to *M. lucianus* and *M. densiflorus* var. *viscidus*, both of which also have very long glandular trichomes relative to the rest of the genus. This is evident in boxplots showing glandular trichome length (Volume 1 Fig. 52). The Vandenberg plants are geographically disjunct from *M. lucianus* by over 200 km and from *M. densiflorus* var. *viscidus* by over 475 km. The Vandenberg plants are geographically closest to *M. fasciculatus* var. *nuttallii*, which also has been documented at Vandenberg and which they appear to hybridize with.

In phylogenetic analyses, the Vandenberg plants are always monophyletic in analyses without hybrids (Figs. 4 and 6). Analyses including putative hybrids with *M. fasciculatus* var. *nuttallii* provide evidence of hybridization as the hybrid plants form a clade with all of the Vandenberg plants plus some of *M. fasciculatus* var. *nuttallii* (Fig. S2).

Based on the combination of evidence presented, the Vandenberg plants are described as the new species *Malacothamnus eastwoodiae* K.Morse below.

MALACOTHAMNUS EASTWOODIAE K.Morse, sp. nov. TYPE: USA. California: Santa Barbara County: Vandenberg Space Force Base: SW quadrant of Snap Rd. and 15th St., 22 June 1987, *Austin P. Griffiths 18245* (holotype: OBI, isotypes: CDA, RSA, UCD, UCR).
Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325439-1

Shrubs 0.5–3 m tall, spreading by rhizomes, stellate trichomes with and without stipes mixed and ± equally abundant. **Stems** with moderate to dense stellate trichomes, stellate trichome rays 1.4 mm or less (mean per plant ranges from 0.4–0.8 mm), stipes ≤ 0.6; glandular trichomes ≤ 0.8 mm (mean per plant ranges from 0.1–0.5 mm); **Leaves** ± round to widely ovate, length generally ≥ width, obscurely to moderately 3–7 lobed, bases cordate, reduced in size distally, subtending two to all glomerules in the inflorescence; generally bright green adaxially and paler abaxially; abaxial stellate trichome density 1–2x adaxial, stellate trichome rays ≤ 1 mm (mean 0.5 mm), stipes ≤ 0.3; glandular trichomes ≤ 0.6 mm (mean per plant ranges from 0.1–0.3 mm). **Inflorescences**: spikes of interrupted and loosely-flowered glomerules, rarely with more proximal branches and/or pedicels elongated; stipular bracts linear, subulate, or falcate, 4–7 mm long, ≤ 1.5 mm wide; calyx bracts linear, 4–11 mm long, 0.3–0.5 mm wide, generally 1/2 to 2/3 of the calyx length, partially to fully red. **Flowers:** calyx not to slightly angled in bud, mature calyx 8.5–15.5 mm long, lobes 5–10 mm long x 2.5–4.5 mm wide, lobe at base 2–4 mm wide, lobe widest at base or up to 2 mm above base, lobe triangular to ovate, lobe tip often reddish, abaxial calyx stellate trichome rays 0.1–2.0 mm (mean per plant ranges from 0.5–0.8 mm), stipe ≤ 0.4; abaxial calyx glandular trichomes 0.1–0.9 mm (mean per plant ranges from 0.3–0.7 mm); corolla pale to deep pink, drying closed, petals to ~3.5 cm, asymmetrically obovate with apex rounded, staminal column 5–9.5 mm. **Fruit:** mericarp 2.2–3.2 mm long by 1.8–3 mm wide, length 1–1.5x width. **Phenology:** peak bloom in May and June, possibly extending into July, a few flowers were observed in August after most fruit dropped. Figure 34 illustrates the inflorescence, calyx, and calyx bracts. All measurements are from dry specimens except shrub height.

Distribution and Habitat—*Malacothamnus eastwoodiae* is currently confirmed from only a small area on Vandenberg Space Force Base in Santa Barbara County, CA south of New Mexico Avenue and north of Terra Road from ~15–120 m in elevation. Smith (1998) notes it is also on Shuman Grade near Casmalia ~16 km from the rest. Smith also notes on specimen SBBG72788 that he had collected similar plants in Lake Canyon without flowers. I have, however, found no vouchers of any *Malacothamnus* from either Shuman Grade or Lake Canyon to confirm and 2021 surveys in these areas found no *Malacothamnus*. *Malacothamnus eastwoodiae* has been found growing in sagebrush scrub, chaparral, and an oak/willow riparian area. Commonly associated species include *Artemisia californica* Less., *Salvia mellifera* Greene, *Baccharis pilularis* DC., *Toxicodendron diversilobum* (Torr. & A.Gray) Greene, and *Acmispon glaber* (Vogel) Brouillet.

Etymology—*Malacothamnus eastwoodiae* is named after Alice Eastwood who described 11 taxa currently included in *Malacothamnus*, which is more than twice that of any other author. Ten of these 11 taxa were not recognized by Bates. I recognize seven taxa Eastwood described, all of which have a rare plant ranking in California, though two are given names by Jepson in this treatment as he described the same taxa shortly after Eastwood using the rank of variety instead of species. Eastwood is also notable for her somewhat poetic

protologs using such descriptors as lovely, delicious, decked with, and snowy (Eastwood 1936). To honor this, I propose the common name Alice's lovely bushmallow as she uses the word "lovely" six times in her treatment of the genus.

Notes— Only one other *Malacothamnus* taxon (*M. fasciculatus* var. *nuttallii*) is known to occur near the range of *M. eastwoodiae. Malacothamnus fasciculatus* var. *nuttallii* is easily distinguished from *M. eastwoodiae* by generally having a panicle-like inflorescence and having much shorter stellate trichomes (0.1–0.2 mm), whereas *M. eastwoodiae* has a spike-like inflorescence and many stellate trichome rays ≥ 0.5 mm. Morphologically-intermediate plants that appear to be hybrids between these taxa are currently known from only a single location. *Malacothamnus eastwoodiae* is morphologically distinguished from the rest of the genus by a combination of several characters. Only three taxa in the genus have both glandular trichomes on the calyx averaging ≥ 0.3 mm and glandular trichomes on the stem averaging ≥ 0.2 mm: *M. eastwoodiae*, *M. lucianus*, and *M. densiflorus* var. *viscidus*. *Malacothamnus eastwoodiae* is distinguished from *M. lucianus* by having a spike-like inflorescence whereas *M. lucianus* has a capitate to subcapitate inflorescence. Distinguishing *M. eastwoodiae* from *M. densiflorus* var. *viscidus* well illustrates the problem of writing an adequate key to the genus without resorting to geographic range. While the means of many measurements are quite different between these two species and the plants can easily be distinguished by the combination of many characters, all measurements in this study overlap at least to some degree. Beyond using geographic range and only using a few characters, *M. eastwoodiae* is most easily distinguished from *M. densiflorus* var. *viscidus* by having relatively dense stellate trichomes on the stem and reddish colored calyx bracts (and often calyx lobe tips) whereas *M. densiflorus* var. *viscidus* has sparser stellate trichomes on the stem and rarely has reddish coloration on the calyx bracts or calyx lobe tips.

Malacothamnus eastwoodiae is a rare species currently confirmed from an area only ~2.5 km^2 (~7 km^2 if the apparent hybrid population is included), which is an area smaller than each of the full ranges of the federally listed *M. fasciculatus* var. *nesioticus* and *M. clementinus*. In 2019 and 2021 surveys, as many as a few thousand plants were observed. However, as *M. eastwoodiae* appears to spread abundantly by rhizomes, it is difficult to estimate how many genetically distinct individuals rather than clones may actually occur in this area. As *Malacothamnus* generally germinate after fire, more plants could possibly be found in postburn surveys nearby. The most recently recorded fire in the known range of this species was in 1941 and only occurred in part of the known range. This suggests the fire history may be incomplete, the range of this species may be larger, the currently known plants may be very old, and/or this species of *Malacothamnus* may be less tied to a fire-following life cycle than others in the genus. Based on its very small range, it is recommended *M. eastwoodiae* be considered for a California Rare Plant Rank of 1B. Vandenberg Space Force Base manages plants of this rank at the ecosystem level with its Integrated Natural Resource Management Plan and the habitat of *M. eastwoodiae* includes several other species of concern already managed under this plan (L. Lum, Vandenberg botanist, pers. comm). Assuming no development is planned for areas where *M. eastwoodiae* occurs, the primary threat to the species may be hybridization, which is well documented as possible between many species by Bates (1963). Only a single small population of apparent hybrids between *M. eastwoodiae* and the other local taxon *M. fasciculatus* var.

nuttallii was found. This population included only morphologically intermediate plants, some more similar to each parent taxon. The main hybridization concern would be the introduction of other *Malacothamnus* taxa including *M. fasciculatus* var. *nuttallii* for restoration or horticultural purposes near populations of *M. eastwoodiae*, which could cause genetic swamping and thus the possible loss of the species. Another possible threat is invasive plants, which may compete with *Malacothamnus* seedlings germinating post-fire.

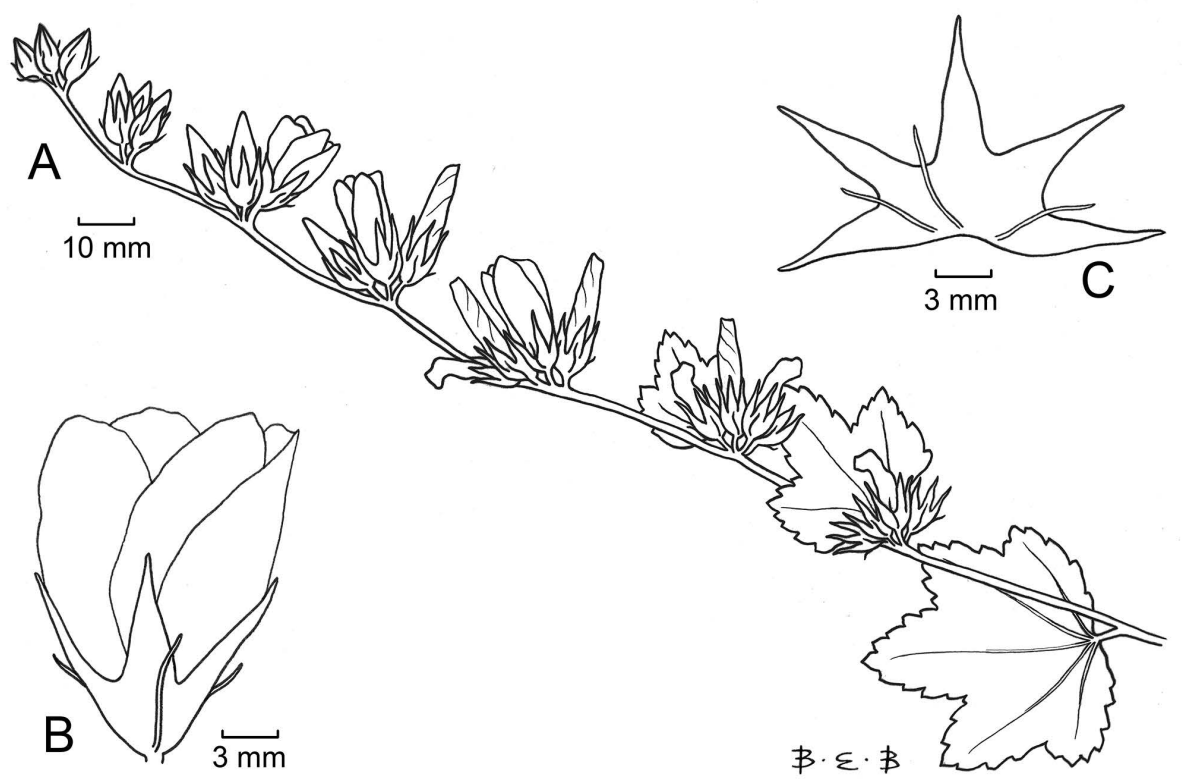

Figure 34. Illustration of *Malacothamnus eastwoodiae*. A. Inflorescence. B. Side view of flower and calyx bracts. C. Dissected and flattened calyx with calyx bracts. Illustration by Brittany E. Burgard.

6.2 *Malacothamnus astrotentaculatus*

Malacothamnus plants from Shasta County, CA were first vouchered in 1949 from a burn plot near the town of Ono. Slotta (2004) is the only reference I found indicating that the Shasta County plants may be different from previously described species. Slotta only examined a single specimen from Shasta County and states nothing about it beyond listing the identification as "Unknown".

The plants from Shasta County are clearly distinct in morphological analyses. They are separated in PCAs including the rest of *M. fremontii* sensu Bates (Volume 1 Figs. 33 and 34) based on calyx lobe shape. This is well illustrated in boxplots of the full genus showing the ratio of the width of the widest part of the calyx lobe to the width at the base (Volume 1 Fig. 47B) where the Shasta County specimens stand out with a high ratio as do *M. aboriginum* and coastal *M. foliosus* sensu Bates. In an LDA of the full genus (Volume 1 Table 18), the Shasta County plants had only one misidentification, this to *M. fremontii* s.s., which the Shasta County plants were shown to be distinct from in PCA. A morphological distance matrix of the full genus (Volume 1 Fig. 44) indicates the Shasta County plants are not strongly similar to any other group. The Shasta County plants are the northernmost known plants in the genus and only geographically near the most northern plants of *M. fremontii* s.s. The closest known *M. fremontii* s.s. to the Shasta County plants is ~40 km away. In phylogenetic analyses, the Shasta County plants are always monophyletic and generally sister to *M. mendocinensis* (Figs. 5 and 6).

Based on the combination of evidence presented, the Shasta County plants are described as the new species *Malacothamnus astrotentaculatus* K.Morse below.

MALACOTHAMNUS ASTROTENTACULATUS K.Morse, sp. nov. TYPE: USA. California: Shasta County: Burned riparian woodland on south side of Platina Rd. between Platina and Ono, 40.4203°, -122.7446°, 412 m, 4 June 2018, *Keir Morse M198* (holotype: RSA898960, isotypes: not yet distributed).
Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325438-1

Shrubs 0.5–1.25 m tall, apparently not or rarely spreading by rhizomes, stellate trichomes mostly without stipes. **Stems** with sparse to dense stellate trichomes, stem surface often visible through trichomes, stellate trichome rays ≤ 1 mm (mean per plant ranges from 0.2–0.4 mm), stipes ≤ 0.5 mm; glandular trichomes ≤ 0.1 mm. **Leaves** ± round to widely ovate, length ±= width, obscurely to moderately 3–7 lobed, bases cordate, reduced in size distally, subtending three to most glomerules in the inflorescence; medium to light green adaxially and paler abaxially; abaxial stellate trichome density 1–2x adaxial, stellate trichome rays ≤ 0.7 mm (mean 0.3 mm), stipe ≤ 0.1 mm; glandular trichomes < 0.1 mm. **Inflorescences:** spikes of conspicuously interrupted dense glomerules; stipular bracts linear, subulate, or falcate, 3–10 mm long, ≤ 1.5 mm wide; calyx bracts linear, 6–11 mm long, 0.2–0.5 mm wide, 2/3 to slightly greater than calyx, green to partially red. **Flowers:** calyx strongly plicate, angled, or winged in bud; mature calyx 8–14 mm long, lobes 5–10 mm long x 5–7 mm wide, lobe at base 2–4 mm wide, lobe widest 1–3.5 mm above base, lobe widely ovate to orbicular with apex abruptly acuminate, abaxial calyx veins with filiform and occasionally somewhat branched outgrowths to ~2.5 mm long covered in stellate trichomes, abaxial calyx stellate trichome rays 0.1–1.2 mm (mean per plant ranges from 0.3–0.5 mm), stipe ≤ 0.5 mm, abaxial calyx glandular trichomes

<0.1 mm; corolla pale to deep pink, generally drying at least somewhat open; petals to ~1.5 cm, asymmetrically obovate with apex rounded, emarginate, or erose; staminal column 3–4.5 mm. **Fruit:** mericarp 2.5–3 mm long by 1.6–2.3 mm wide, length 1.3–1.8x width. **Phenology:** peak bloom within its geographic range apparently in June with blooming also likely in May and possibly extending into July, a few flowers were observed in August on plants mostly in fruit. Plants grown at California Botanic Garden bloomed in May and June. Figure 35 illustrates the inflorescence, glomerule in bud, calyx, and calyx bracts. All measurements are from dry specimens except shrub height.

Distribution and Habitat— *Malacothamnus astrotentaculatus* is currently only known to occur in Shasta and Tehama counties in CA from ~400–1070 m in elevation. It has been found growing in chaparral, oak woodlands, pine/oak woodlands, and riparian woodlands that have burned recently. This species ranges from Beegum Gorge Road on the west end to Hirz Mountain Lookout on the northeast end. The southernmost confirmed location is ~4.5 km south of Platina and a poorly photographed observation indicates it may occur at least 14 km south of Platina. Common associated taxa include: *Adenostoma fasciculatum* Hook. & Arn., *Arctostaphylos spp*. Adans, *Ceanothus cuneatus* (Hook.) Nutt., *Eriodictyon californicum* (Hook. & Arn.) Torr., *Eriophyllum lanatum* (Pursh) J.Forbes, *Pinus sabiniana* Douglas, *Quercus chrysolepis* Liebm., *Quercus garryana* Douglas ex Hook., and *Toxicodendron diversilobum*. Recent collections were all made within 11 years after an area burned. Only a single plant was found in the area burned 11 years prior. All other recent collections were from within 5 years after an area burned.

Etymology—*Malacothamnus astrotentaculatus* is named after the distinctive outgrowths on the calyx veins that somewhat resemble tentacles (Fig 36). As these "tentacles" are covered with stellate trichomes, the common name starry-tentacled bushmallow and its translation to *M. astrotentaculatus* becomes almost obligatory. Not only can starry tentacles be seen on the calyx, but the distinctive glomerules of this species resemble clusters of stars that sway in the breeze on tentacle-like branches, which makes this species doubly astrotentacular.

Notes— No other *Malacothamnus* species are currently known to occur within the range of *M*. *astrotentaculatus.* Morphologically, *M. astrotentaculatus* is distinguished from all other species of *Malacothamnus* by the combination of several characters. The most prominent character is the shape of the calyx lobes. The widest area of the calyx lobe is ~1.5–3 times the width at the calyx lobe base. With the exception of *M. aboriginum* and *M. foliosus* sensu Bates, the widest area of the calyx lobe of other species of *Malacothamnus* does not or rarely exceeds 1.5 times the width at the calyx lobe base. Due to this shape, the edges of the calyx lobes stick out in bud making the buds appear strongly angled or winged in *M. astrotentaculatus*, *M. aboriginum*, and the more coastal forms of *M. foliosus* sensu Bates. The flowers are clustered on spike-like inflorescences in *M. astrotentaculatus* and *M. aboriginum* whereas the inflorescences of the more coastal forms of *M. foliosus* sensu Bates are open panicles. While *M. astrotentaculatus* does look somewhat similar to *M. aboriginum*, it can be distinguished based on the width of the calyx bracts. The calyx bracts of *M. astrotentaculatus* are ≤ 0.5 mm wide whereas in *M. aboriginum* they are ≥ 1.7 mm. An interesting character not measured for these analyses that sets *M. astrotentaculatus* apart from other species in the genus are outgrowths on the most prominent veins on the abaxial surface of the calyx that are filiform and sometimes

somewhat branched. These outgrowths are covered in stellate trichomes like much of the rest of the plant (Fig. 36). I have found a few of these outgrowths in other species, on just one specimen each of *M. foliosus* sensu Bates (coastal form), *M. howellii*, and *M. fremontii* s.s., as well as on a few specimens of *M. palmeri*. While the outgrowths are not limited to *M. astrotentaculatus*, this is the only species I have observed them on all specimens I have examined and where they are relatively easy to detect. Note that these outgrowths are very fragile on dry specimens, so perhaps are best detected by probing on fresh or rehydrated specimens.

Malacothamnus astrotentaculatus is a rare species known to occur in as many as six areas and documented only in four areas since 2017. Approximately 100 plants were found in an area between Platina and Ono centered on Duncan Creek Rd. (the type locality), which burned five years before observation, though additional plants are likely to occur there as much of the burned area was not surveyed. A single plant was found along Beegum Gorge Rd., which burned 11 years before observation. A nearby historical collection and abundant potential habitat indicate additional plants may be found in that area in years closer to a burn before dense chaparral grows back. The only confirmed population in Tehama County with ~1000 plants was found in 2022, one year after the area burned, along Tedoc Road south of Platina. A fourth location of ~500 plants was found in 2021 near Hirz Mountain Lookout three years after the area burned. Despite the likelihood of more plants being found in the future, with very few plants being currently known, it is recommended that *M. astrotentaculatus* be considered for a California Rare Plant Rank of 1B. Beyond the usual threats associated with a species having low numbers of plants, another possible threat may be postfire vegetation treatments. An area of burned chaparral adjacent to one location of *M. astrotentaculatus* was observed to have been treated with herbicide postfire and planted with pines. As *Malacothamnus* generally germinate after fires and as many often only live a few years before being outcompeted by returning chaparral, they may only have a small amount of time to replenish the soil seedbank. If a burned area were given a postburn vegetation treatment that prevented *Malacothamnus* from reaching maturity and replenishing the soil seedbank, it may reduce or eliminate the potential for it to recover naturally in that area.

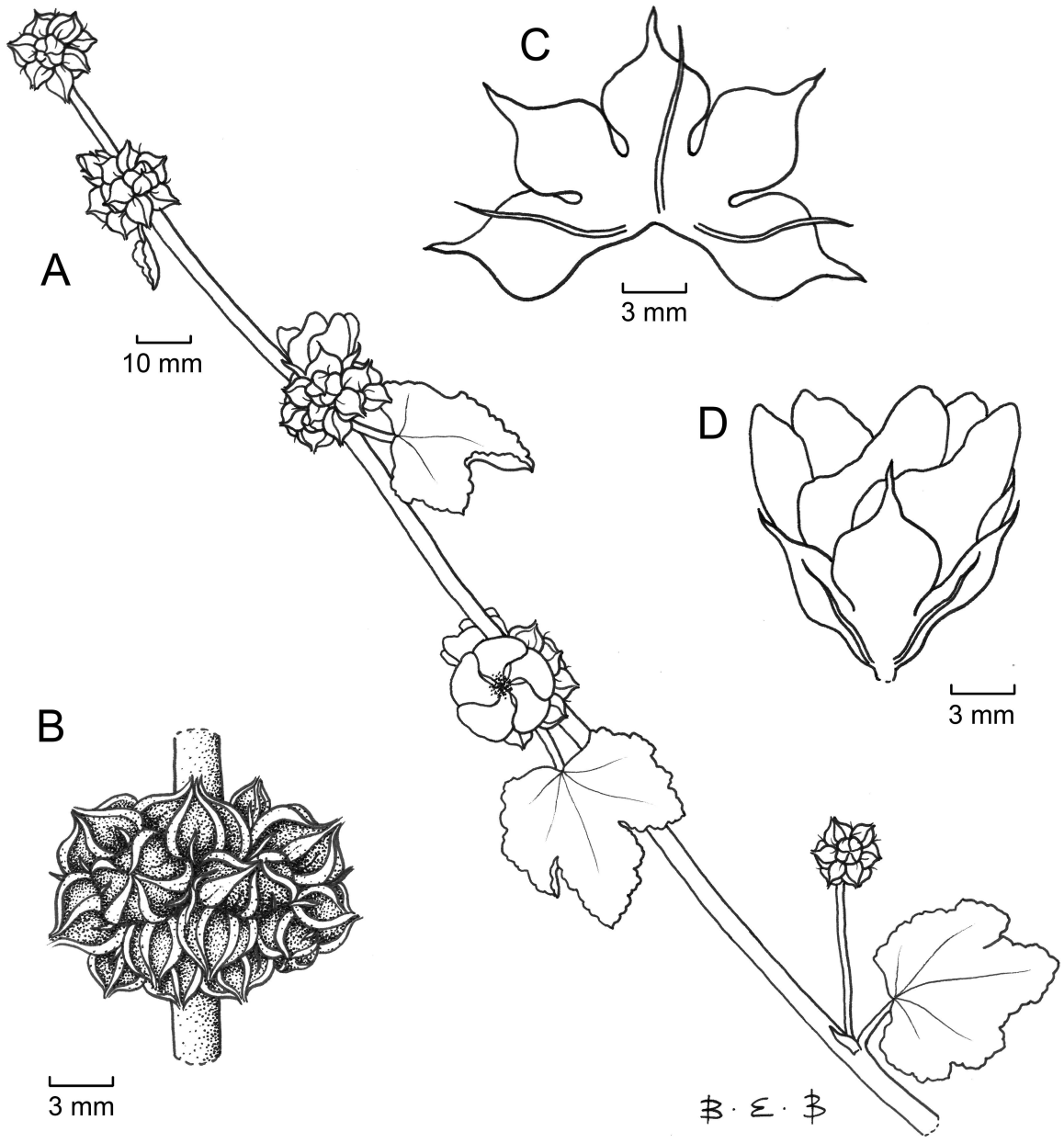

Figure 35. Illustration of *Malacothamnus astrotentaculatus*. A. Inflorescence. B. Glomerule in bud showing protruding calyx lobe margins. C. Dissected and flattened calyx with calyx bracts. D. Side view of flower with calyx bracts. Illustration by Brittany E. Burgard.

Figure 36. SEM image of one of the filiform to somewhat branched calyx vein outgrowths covered in stellate trichomes found on all *Malacothamnus astrotentaculatus* specimens examined. This star-studded tentacle-like outgrowth is the inspiration for the species name.

6.3 *Malacothamnus discombobulatus*

What has been called *Malacothamnus davidsonii* in previous treatments occurs in two regions of California disjunct by ~330 km. The southern region of plants occurs in Los Angeles County (the type locality) and the northern region occurs in Monterey and San Luis Obispo counties. Plants from both regions are morphologically similar and bloom relatively late relative to most other taxa in the genus, which provides evidence as to why they could be considered a single species. Assuming they were closely related, the large geographic disjunction would mean that there has been no recent geneflow between the two regions. This could lead to divergence, possibly partly due to geneflow with nearby taxa, that would perhaps be enough to recognize plants from each region as varieties of a single species or as distinct species. An alternate hypothesis would be that the plants from these two regions are not closely related and that their morphological and phenological similarity is due to convergent evolution.

Morphological evidence shows plants from the two regions of *M. davidsonii* to be very similar, though significantly different from a statistical point of view (Volume 1 Chapter 3.3). Phylogenetic analyses, however, show plants from these two regions to be highly divergent with each more closely related to taxa they co-occur with than to each other (Figs. 3, 5, 20, and 21). Population-level sampling of both regions of *M. davidsonii* plus the taxa they co-occur with could provide evidence of whether these two regions of *M. davidsonii* are a relatively recent divergence from a single taxon or a morphological convergence of unrelated taxa. Whichever reason for their similarities is correct, the current evidence indicates that they are not closely related to each other and that the northern region should be recognized as a separate species.

Based on the combination of evidence presented, plants of what have been previously called *M. davidsonii* from Monterey and San Luis Obispo counties are described as the new species *Malacothamnus discombobulatus* K.Morse below.

MALACOTHAMNUS DISCOMBOBULATUS K.Morse, sp. nov. TYPE: USA. California: San Luis Obispo County: On both sides of Interlake Road ~2.2 km west of its intersection with Nacimiento Lake Drive, 35.7777°, -120.9145°, 440 m, 15 August 2018, *K. Morse M266* (holotype: RSA899224, isotypes: not yet distributed).
Life Science Identifier (LSID): urn:lsid:ipni.org:names:77325440-1

Shrubs up to 5 m tall, occasionally spreading by rhizomes (possibly rarely), stellate trichomes not dominated by those with or without stipes. **Stems** with moderate to dense stellate trichomes, stem surface sometimes visible through trichomes distally, stellate trichome rays ≤ 0.7 mm (mean per plant ranges from 0.2–0.5 mm), stipes ≤ 1.1 mm; glandular trichomes < 0.1 mm. **Leaves** ± round to widely ovate, length ≥ width, obscurely to moderately 3–7 lobed, bases cordate, reduced in size distally, subtending one to all nodes in the inflorescence, pale to bright green adaxially and often paler abaxially; abaxial stellate trichome density 0.7–1.6x adaxial, stellate trichome rays ≤ 0.6 mm (mean 0.3 mm), stipe ≤ 0.3 mm; glandular trichomes < 0.1 mm. **Inflorescences** narrowly to widely panicle-like, occasionally spike-like; stipular bracts

lanceolate, triangular, ovate, or widely ovate, occasionally shallowly to deeply 2–4 lobed, 1.5–4 mm long, ≤ 2.5 mm wide; calyx bracts linear to lanceolate, length 3–10x longer than width, 1–3 mm long, 0.3–0.6 mm wide, 1/10 to 2/5 of calyx length, green to partially or fully red. **Flowers**: calyx not to slightly angled in bud, mature calyx 6.5–9.5 mm long, lobes 4–6 mm long x 2–2.5 mm wide, lobe at base 2–2.5 mm wide, lobe widest at base or up to 1.5 mm above base, lobe narrowly to moderately triangular or ovate with acuminate apex; abaxial calyx stellate trichome rays 0.1–0.5 mm (mean per plant ranges from 0.15–0.3 mm), stipe ≤ 0.4 mm; abaxial calyx glandular trichomes < 0.1 mm; corolla pink, drying closed, petals to ~1.7 cm, asymmetrically obovate with apex rounded, staminal column 3–5 mm. **Fruit**: mericarp 2.3–2.6 mm long by 1.3–1.8 mm wide, length 1.4–2x width. **Phenology**: blooming June to October (possibly earlier or later) with peak bloom in July and August, later blooms are generally sporadic flowers in the already fruited inflorescences.

Distribution and habitat—*Malacothamnus discombobulatus* occurs in Monterey and San Luis Obispo counties in California west of the Salinas River, south of King City, north of Lake Nacimiento, and east of the most coastal mountains. Habitats include coastal scrub, chaparral, oak savannah, and riparian woodland. Associated species are diverse and vary between sites. Like all or most of the genus, *Malacothamnus discombobulatus* presumably mostly germinates after fire and is relatively short-lived. One record notes that one year after an area burned this species was locally dominant with thousands of plants found and another record notes "prevalent in recent burn" (CDFW 2022).

Etymology—*Malacothamnus discombobulatus* is named after the confusion sown from its morphological similarity to *M. davidsonii* and to the pending future discombobulation when someone inevitably includes these two species together in a misguided restoration planting, assuming that hasn't already happened. I propose the common name discombobulating bushmallow, which is almost but not quite as fun to say as *Malacothamnus discombobulatus*.

Notes—*Malacothamnus discombobulatus* is morphologically most similar to *M. davidsonii*, which it can be very difficult to distinguish from. Assuming no plants from one species have been planted into the range of the other, the easiest way to distinguish them is using their geographic ranges. Outside of cultivation, *Malacothamnus discombobulatus* is only known to occur in Monterey and San Luis Obispo counties while *M. davidsonii* is only known to occur in Los Angeles County. For those who wish to attempt to distinguish them morphologically, ~90% of specimens of *M. davidsonii* examined could be distinguished from *M. discombobulatus* by one or more of the following characters based on measurements of dry specimens: calyx lobe width ≥ 3 mm, calyx lobe length < 4 mm, calyx lobe length/width ≤ 1.5, and some glandular trichomes on the stem or calyx ≥ 0.1 mm. As can be deduced by those measurements, the calyx lobes of *M. discombobulatus* are often, but not always, longer and narrower than those of *M. davidsonii* as seen in Figure 37.

Figure 37. Comparison of calyces of *M. discombobulatus* (left) and *M. davidsonii* (right) grown at California Botanic Garden, which represent relatively distinct morphological forms of each species. The calyx lobes of *M. discombobulatus* are often, but not always, longer and narrower than those of *M. davidsonii*.

Populations of *Malacothamnus discombobulatus* have been included in rare plant assessments of *M. davidsonii* in the past (CNPS 2022). *Malacothamnus davidsonii* (including *M. discombobulatus*) has a California Rare Plant Rank (CRPR) of 1B.2. A CRPR rank of 1B.2 indicates plants that are rare, threatened, or endangered in California and elsewhere, which are moderately threatened in California with 20-80% of occurrences threatened. The current known range of *M. discombobulatus* is ~450 km^2. Assessing population sizes is difficult as plants may be short-lived and dormant in the soil seed bank until an area burns. As noted previously, one population had thousands of plants post-fire. Twelve other populations have size estimates ranging from 1 to 250 plants. Beyond the usual threats associated with a species having a narrow geographic range and a relatively small population size, other threats to its long-term status may include competition with invasive species when seeds germinate post-fire and possibly hybridization with misidentified or poorly chosen *Malacothamnus* species introduced for restoration purposes, which I have encountered multiple times in other parts of California. In particular, introduction of *M. davidsonii* into the range of *M. discombobulatus* or vice-versa would be difficult to detect and remedy before the possible damage of hybridization is done.

7 CONCLUSIONS

7.1 Taxonomic Conclusions

Assessing the *Malacothamnus* morphological groupings from Volume 1 (Morse 2023) using RAD-seq data greatly clarifies the relationships between some groups and identifies which groups are in most need of further research. Thirty-six of the 38 morphological groups were assessed in this study providing various levels of phylogenetic evidence to assist in taxonomic decisions. Based on all currently available evidence, I recognize 29 minimum rank taxa within 21 *Malacothamnus* species (Table 1). One new morphologically cryptic species, *M. discombobulatus*, is described based primarily on phylogenetic and geographic evidence. Two morphologically distinct new species, *M. astrotentaculatus* and *M. eastwoodiae*, are described based on the combination of morphological, geographic, and phylogenetic evidence. Four taxa recognized for the first time at the rank of variety within *Malacothamnus* needed new combinations or ranks: *M. fremontii* var. *exfibulosus*, *M. arcuatus* var. *elmeri*, *M. jonesii* var. *gracilis*, and *M. jonesii* var. *niveus*. Those taxa I have chosen to recognize at the rank of variety all could arguably be treated at the species rank, but I have chosen a somewhat tempered approach to allow some morphologically ambiguous plants to be identified to a single species rather than as intermediates, especially in groups where more research is needed. These 29 taxa are used in a new *Malacothamnus* treatment in Volume 3 of this monograph.

Of the 29 *Malacothamnus* taxa I recognize, 15 are listed as taxa of conservation concern in California (CNPS 2022). Nine of these taxa of conservation concern were not recognized in the Flora of North America treatment (Bates 2015) and five were not recognized in the most recent Jepson Manual treatment for California (Slotta 2012). I additionally recognize four new species with limited distributions, one described in a previous article (Morse and Chester, 2019), that have not yet been considered for a conservation status. With the addition of these four new species, there are at least 13 taxa of possible conservation concern not recognized in Bates's treatments (1993, 2015), which is more taxa than he recognized in the entire genus. This highlights taxonomic decisions as a possible major threat to rare taxa. Taxa not recognized in treatments can be easily overlooked when identifying plants and the lack of recognition may prevent them from being protected by environmental regulations or lead to decisions that could be detrimental to some taxa. To help provide some data for conservation decisions, preliminary conservation assessments of all 29 taxa are included in Volume 3 with the treatment.

7.2 Future Research Needs

While this study provides a good baseline for assessing the phylogenetic relationships between *Malacothamnus* taxa, apparent gene flow and/or incomplete lineage sorting between some groups leaves many questions. This study used broad geographic sampling of the entire genus with rarely more than one sample per population. To assess gene flow between populations, a higher level of sampling per population is needed. Like the deficiency in

specimen sampling, the RAD-seq approach resulted in a high amount of missing data in samples relative to each other, which can be problematic for analyses requiring a full data matrix. A targeted capture approach resulting in a more uniform dataset may yield clearer results in taxa that were problematic using RAD-seq. This approach may also allow for sampling of the smaller DNA fragments of older type specimens such as *M. foliosus*, *M. paniculatus*, *M. parishii*, and the *M. fasciculatus* synonym *Malvastrum thurberi* A.Gray, which could help resolve questions related to these taxa. The types of both *M. foliosus* and *M. paniculatus* are morphological outliers in analyses of *M. foliosus* s.l., *M. parishii* is a presumed extinct species that is likely a morphological extreme of *M. fasciculatus* var. *laxiflorus*, and the purported type locality of *Malvastrum thurberi* is disjunct from the rest of the genus.

Regarding taxonomy in *Malacothamnus*, three groups of taxa would possibly benefit the most from future research: (1) the *M. fremontii* clade, (2) the varieties of *M. fasciculatus* plus *M. parishii*, and (3) the *Malacothamnus* of Baja California and San Diego County.

Malacothamnus fremontii s.s. was poorly sampled in this study due to large geographic gaps between known populations. Additional sampling including plants from these gaps, if they can be found, may help clarify the relationships between the possibly intermediate northern populations of *M. fremontii* s.s. and the rest of the *M. fremontii* clade. Population-level sampling may also help clarify the relationship between *M. fremontii* s.s. and *M. howellii*, which I am tentatively treating as synonyms.

Malacothamnus fasciculatus var. *laxiflorus* is polyphyletic in this study and plants from the western region of this variety, including the type locality, may be somewhat intermediate between the other varieties of *M. fasciculatus*. Population-level sampling of the *M. fasciculatus* varieties is likely needed to resolve the relationships between them and could possibly provide evidence to recognize all or some as species. *Malacothamnus parishii,* which I suspect is an extreme form of *M. f.* var. *laxiflorus,* should be assessed with the *M. fasciculatus* varieties as well. If eastern *M. f.* var. *laxiflorus* is distinct from the western regions, as possibly indicated by this study, but not distinct from *M. parishii*, the eastern *M. f.* var. *laxiflorus* would likely need to be treated as a more broadly defined *M. parishii* as the type specimen of *M. f.* var. *laxiflorus* is from the possibly not closely related western regions.

Relationships between plants from Baja California and adjacent San Diego County (the southern portion of the southern clade in Figure 2) were the most poorly resolved in the genus from a phylogenetic standpoint. This may be due to gene flow and/or incomplete lineage sorting between taxa but is also likely due to limited sampling in Baja California. There are no *Malacothamnus* specimens from many large regions of Baja California, which may hold variation currently unaccounted for. Some of these regions not sampled have iNaturalist observations of “Baja East”, which I have tentatively placed in *M. enigmaticus* and needs further research. Parts of the range of *M. foliosus* are currently well surveyed on iNaturalist but none of the observations match the morphology of the type collection and large areas without voucher specimens or iNaturalist observations are immediately adjacent to well-surveyed

areas. Plants from Baja California should be assessed further, especially relative to plants from San Diego County, but additional surveys and sampling in Baja California should be implemented to better document the variation and ranges of taxa there before additional phylogenetic work is undertaken.

Data Repositories

Raw data available from SRA Genbank: PRJNA928782
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA928782

Trees and SNP matrices used to generate them available from FigShare:
https://doi.org/10.6084/m9.figshare.23982744

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Appendix 1 - Specimens Sampled

Herbarium voucher specimens representing populations tissue used in this study was collected from are listed alphabetically by *Malacothamnus* taxon followed by suspected hybrids and outgroups. Within each, they are organized by location to the county level, then alphanumerically by collector and collection number. Accession number of accessioned specimens are in parentheses. Specimens not yet accessioned are marked with TBD (to be determined) in parentheses. At least one sheet of each TBD collection will be housed at RSA. An asterisk indicates a cultivated specimen or suspected planting. A tilde indicates specimens excluded from analyses due to too few recovered loci. UCBG samples are unvouchered collections from living accessions at University of California Berkeley Botanic Garden. SBBG samples are from the Santa Barbara Botanic Garden Tissue Bank, most of which are also vouchered in the SBBG herbarium.

Malacothamnus abbottii (Eastw.) Kearney. USA. California: Monterey County: *K. Morse M113, M270, M271* (TBD). Los Angeles County: *K. Morse M285*, M474*[*RA1*]*** (TBD).

Malacothamnus aboriginum (B.L. Rob.) Greene. USA. California: Fresno County: *K. Morse M240* (TBD). Los Angeles County: *K. Morse M474* (TBD). Monterey County: *K. Morse M180* (TBD). San Benito County: *K. Morse M110, M177, M381* (TBD*).*

Malacothamnus arcuatus (Greene) Greene var. ***arcuatus***. USA. California: Alameda County*: J. T. Howell 12579* (CAS221806). San Mateo County: *K. Gallagher 2021-01* (TBD); *K. Morse M175, M208* (TBD). Santa Clara County: *E. Bond s.n. 15 Jun 1928* (POM175447~), *K. Morse M174* (TBD), C. Wolf s.n. 1929-06-08 (RSA13290~). Santa Cruz County: *D. Neubauer & A. Patten 1966* (UCSC).

Malacothamnus arcuatus (Greene) Greene var. ***elmeri*** (Jeps.) K.Morse. USA. California: Contra Costa County: K. Morse M187 (TBD). Merced County: *K. Morse M108* (TBD). Santa Clara County: *J. Mo s.n. 30 May 2018* (TBD), *K. Morse M176, M181, M377b, M378* (TBD).

Malacothamnus astrotentaculatus K.Morse. USA. California: Shasta County: *K. Morse M194, M195, M196, M198, M410* (TBD).

Malacothamnus clementinus (Munz & I.M. Johnst.) Kearney. USA. California: Los Angeles County: *S. Calloway SCI-31, SCI-42* (SBBG); *A. Liston 76-1* (germinated seed from seedbank collection vouchered as RSA515088~); *B. Munson BM1~, BM2, BM3, BM4, BM5, BM6, BM7* (TBD).

Malacothamnus davidsonii (B.L. Rob.) Greene. USA. California: Los Angeles County: *K. Morse M118, M143, M258, M259, M315, M316, M317, M420* (TBD).

Malacothamnus densiflorus* var. *densiflorus (S. Watson) Greene. USA. California: Riverside County: *K. Morse M046b, M131, M133, M387, M392*. Orange County: *K. Morse M386* (TBD). San Diego County: *K. Morse M009, M050, M069, M070a, M081b, M086, M215, M216, M333, M334, M336, M450, M451* (TBD).

Malacothamnus densiflorus* var. *viscidus (Abrams) Kearney. MEXICO. Baja California: *J. P. Rebman 11843* (SD160852). USA. California: San Diego County: *K. Morse M049, M067a, M068, M071b, M217, M218, M332* (TBD).

Malacothamnus discombobulatus K.Morse**.** USA. California: Monterey County: *K. Morse M268b, M269, M272* (TBD). San Luis Obispo County: *K. Morse M089* (TBD).

Malacothamnus eastwoodiae K.Morse. USA. California: Santa Barbara County: *K. Morse M391, M398, M399, M403, M404* (TBD).

Malacothamnus enigmaticus K.Morse & T.Chester. MEXICO. Baja California: *R. D. Cadena 4122* (SD23448), *R. Moran 13578* (SD63435), *R. Moran 18043* (SD76526), *R. Moran 24049* (SD97104), *R. Moran 27712* (SD103022). USA. California: San Diego County: *T. Chester 2153-1, 2155-1, 2236, 2237, 2238* (TBD); *K. Morse M060b, M061a, M062a, M065c, M081a, M213, M214* (TBD).

Malacothamnus fasciculatus* var. *catalinensis (Eastw.) Kearney. USA. California: Los Angeles County: *K. Morse M339, M340, M341, M342, M343, M344, M345, M346~, M347, M348* (TBD).

Malacothamnus fasciculatus* var. *fasciculatus (Nutt. ex Torr. & A. Gray) Greene. MEXICO. Baja California: *C. F. Harbison 45503* (SD45491~), *R. Moran 11012* (SD54749), *J. J. Perez Navarro 762* (SD141142), *J. P. Rebman 1204* (SD137107), *J. P. Rebman 5107* (SD144264), *J. P. Rebman 5351* (SD145295), *J. P. Rebman 22972* (SD223020), *J. Riley 320* (SD254037), *I. L. Wiggins 366* (SD94552). USA. California: San Diego County: *K. Morse M008, M010, M014, M016, M030, M033, M039, M219, M220, M337* (TBD); *J. P. Rebman et al 20779* (RSA779371).

Malacothamnus fasciculatus* var. *laxiflorus (A. Gray) Kearney. USA. California: Los Angeles County: *A. Davidson s.n. 10 Apr 1890* (RSA434232), *K. Morse M129, M130, M142, M152, M256, M313, M318, M319, M320* (TBD); *M. Tollett s.n. 28 Mar 2021* (TBD). Orange County: *K. Morse M135, M309, M310, M314, M456* (TBD). Riverside County: *K. Morse M044, M055, M209, M210, M211, M324, M328, M338* (TBD). San Bernardino County: *K. Morse M123, M148, M149, M212, M250, M296, M297, M418, M419* (TBD). Ventura County: *K. Gallagher 2018-017* (TBD); *K. Morse M321, M322, M323, M443* (TBD).

Malacothamnus fasciculatus* var. *nesioticus (B.L. Rob.) Kearney. USA. California: Santa Barbara County: *S. Carson ALM7* (SBBG); *K. Hasenstab-Lehman 2162* (SBBG); *J. Knapp s.n. 10 Apr 2018* (SBBG); *K. McEachern s.n. 22 Jul 2020 "Central Valley SBBG 99-231", s.n. 22 Jul 2020 "Horchata SBBG 97-201", s.n. 23 Jul 2020 "Cebada East", s.n. 23 Jul 2020 "Christy Ranch"* (SBBG); *H. Schneider PA1, PA6, PA11* (SBBG); *H. Schneider et. al. ALM1, ALM2* (SBBG).

Malacothamnus foliosus (S. Watson) Kearney. MEXICO. Baja California: *J. Campos Sauzal1* (TBD), *J. Campos Sauzal2* (TBD), *R. F. Hoover 56023* (SD124043), *R. Moran 16025* (SD71522), *R. Moran 16515* (SD71923), *R. Moran 22923* (SD95506), *R. Moran 26120* (SD100579), *R. Moran 29838* (SD109026), *K. Morse & J. Riley M354, M355, M356, M358* (TBD), *I. L. Wiggins 11869* (SD47285).

Malacothamnus fremontii (Torr. ex A.Gray) Greene var. **fremontii**. USA. California: Amador County: *K. Morse M184* (TBD). Calaveras County: *W. R. Howden 235* (RSA121093), *K. Morse M104* (TBD). Contra Costa County: *K. Morse M185, M186, M188, M380* (TBD). Fresno County: *K. Morse M202, M376* (TBD). Mariposa County: *K. Morse & A. Colwell M239* (TBD). Stanislaus County: *K. Morse M183* (TBD). Sutter County: *K. R. Stern 8190* (CHSC41320). Tehama County: *B. Castro & L. P. Janeway 972* (CHSC77603). Tulare County: *K. Morse M103, M134, M203, M373* (TBD). Yuba County: *K. Morse M414* (TBD).

Malacothamnus fremontii (Torr. ex A.Gray) Greene var. ***exfibulosus*** (Jeps.) Morse. USA. California: Colusa County: *K. Morse M105, M191, M192* (TBD). Lake County: *K. Morse M189, M407* (TBD).

Malacothamnus involucratus (B.L. Rob.) K. Morse. USA. California: Monterey County: *C. Bronny s.n. 18 Jun 2021*; *K. Morse M091, M111, M172, M382a* (TBD).

Malacothamnus jonesii (Munz) Kearney var. ***gracilis*** (Eastw.) K.Morse. USA. California: San Luis Obispo County: *K. Morse M117, M157, M158, M245, M246* (TBD).

Malacothamnus jonesii (Munz) Kearney var. ***jonesii.*** USA. California: Monterey County: *K. Morse M162, M382b* (TBD). San Luis Obispo County: *K. Morse M093*, *M094*, *M095*, M273, *M364* (TBD).

Malacothamnus jonesii (Munz) Kearney var. ***niveus*** (Eastw.) K.Morse. USA. California: Monterey County: *K. Morse M160, M161* (TBD). San Luis Obispo County: *G. Butterworth GB2* (TBD); *K. Morse M116, M154, M159, M363* (TBD)*; K. Morse & G. Butterworth M432* (TBD). Santa Barbara County: *K. Morse M362* (TBD).

Malacothamnus lucianus (Kearney) K. Morse. USA. California: Alameda County: *C. Loughran 85.1139** (UCBG). Monterey County: *K. Morse M171* (TBD), *K. Morse et al M169, M170* (TBD), *K. Morse & A. Herman M163, M164, M166* (TBD).

Malacothamnus marrubioides (Durand & Hilg.) Greene. USA. California: *K. Morse M073, M136, M141, M147, M286, M311, M441* (TBD).

Malacothamnus mendocinensis (Eastw.) Kearney. USA. California: Mendocino County: *K. Morse & J. Xerogeanes M408* (TBD), *L. S. Rose s.n. 30 May 1939* (POM259590), *J. Xerogeanes s.n. 20 Sep 2016* (TBD).

Malacothamnus nuttallii Abrams. USA. California: San Luis Obispo County: *K. Morse M405* (TBD). Santa Barbara County: *K. Morse M124, M125, M126, M263, M264, M325* (TBD). Ventura County: *K. Morse M260, M261, M262* (TBD).

Malacothamnus orbiculatus (Greene) Greene. USA. Arizona: Maricopa County: *L. R. Landrum 9388* (ASU227611), *K. Morse M330, M429** (TBD). Mohave County: *G. Clifton 47274* (GLC11397). California: Inyo County: *K. Morse M099, M100, M228, M238* (TBD). Kern County*: N. Jensen 3714, 3756* (TBD); *K. Morse M080, M206, M207* (TBD). Los Angeles County: *K. Morse M097, M127, M140, M146, M295, M368, M370* (TBD). San Bernardino County: *K. Morse M121, M292, M395, M417* (TBD). Santa Barbara County: *K. Morse M153* (TBD). Tulare County: *K. Morse M101, M102* (TBD). Ventura County: *K. Morse M077, M359*.

Malacothamnus palmeri (S. Watson) Greene. USA. California: San Luis County: *D. R. Miller & E. A. Appel 611* (OBI75714), *K. Morse M092, M167* (TBD).

Intermediates of *M. enigmaticus*, *M. fasciculatus* var. *fasciculatus* and/or *M. foliosus*. MEXICO. Baja California: *R. Moran 14542* (SD68186~), *R. Moran 15499* (SD68882~), *R. Moran 18043* (SD76526), *R. Moran 23413* (SD96879).

Intermediates of *M. densiflorus* var. *densiflorus* and *M. fasciculatus* var. *fasciculatus*. USA. California: San Diego County: *A. Herman M035* (TBD); *K. Morse M002, M005, M020* (TBD).

Intermediates of *M. eastwoodiae* and *M. nuttallii*. USA. California: Santa Barbara County: *K. Morse M400, M401, M402* (TBD).

Intermediates of *M. fasciculatus* var. *fasciculatus* and *M. foliosus*. MEXICO. Baja California: *J. Campos KM84* (TBD), *J. Campos KM86.8* (TBD), *A. Carter 3186* (SD176811), *A. Carter 3189* (SD176810~), *R. Moran 24380* (SD97838), *K. Morse & J. Riley M357* (TBD), *J. P. Rebman 3955* (SD144363), *J. P. Rebman et al 22817~* (TBD), *S. Vanderplank CO-6* (SD254038).

Intermediates of *M. fremontii* var. *fremontii* and *M. orbiculatus*. USA. California: Tulare County: *K. Morse & N. House M436, M437* (TBD).

Intermediates of *M. jonesii* varieties. USA. California: San Luis Obispo County: niveus/jonesii intermediate *A. Herman s.n. 13 Jun 2019* (TBD). Santa Barbara County: niveus/gracilis intermediates *K. Morse M155, M156* (TBD).

Intermediates of *M. orbiculatus* and *M. fasciculatus* var. *laxiflorus*. USA. California: Los Angeles County: *K. Morse M350, M353* (TBD). San Bernardino County: *K. Morse M254_1, M254_2, M290* (TBD). Ventura County: *K. Morse M444* (TBD).

Intermediates of *M. orbiculatus*, *M. fasciculatus* var. *laxiflorus,* and/or *M. marrubioides*. USA. California: Los Angeles County: *K. Morse M072, M371* (TBD).

Intermediates of *M. orbiculatus* and *M. marrubioides*. USA. California: Los Angeles County: *K. Morse M289, M427* (TBD).

Additional intermediates/hybrids. USA. California: Alameda County: *M. lucianus/fasciculatus* var. *nesioticus* hybrid? *C. Loughran 65.1187** (UCBG), *M. lucianus/palmeri* hybrid? *C. Loughran 85.0039** (UCBG). Contra Costa County: *M. arcuatus var. elmeri/fremontii* var. *fremontii* intermediate *K. Morse M379* (TBD). Los Angeles County: *M. aboriginum/nuttallii* hybrid *K. Morse M390** (TBD), *M. clementinus/palmeri* s.l. hybrid? *K. Morse M385** (TBD), *M. davidsonii/fasciculatus* var. *laxiflorus* intermediate *K. Morse M425* (TBD). Riverside County: *M. densiflorus/fasciculatus* intermediate? *K. Morse M045a, M096* (TBD). San Diego County: *M. enigmaticus/ densiflorus* var. *densiflorus* intermediate *K. Morse M082* (TBD), *M. enigmaticus/fasciculatus* var. *laxiflorus* intermediate? *K. Morse M063a* (TBD). San Luis Obispo County: *M. marrubioides* hybrid? *K. Morse M361** (TBD). Santa Cruz County *M. lucianus/fasciculatus* var. *nesioticus* hybrid? *K. Morse M173** (TBD).

Outgroups. USA. Arizona: Maricopa County: *Abutilon parvulum K. Morse M305* (TBD), *Sida abutifolia K. Morse M304* (TBD). California: Humbolt County: *Iliamna latibractata K. Morse M411* (TBD). Inyo County: *Sphaeralcea ambigua K. Morse M225* (TBD). Los Angeles County: *Malva parviflora K. Morse M151* (TBD), *Phymosia umbellata K. Morse M422** (TBD). Monterey County: *Sidalcea malviflora* subsp. *laciniata K. Morse M165* (TBD). San Diego County: *Eremalche rotundifolia K. Morse M327* (TBD), *Hibiscus denudata K. Morse M326* (TBD). Siskiyou County: *Iliamna bakeri K. Morse M199* (TBD). Nevada: Elko County: *Iliamna rivularis M. Berger s.n. 25 Jun 2021* (TBD).

Appendix 2 - DNA Extraction Protocol

Note: 3M Sodium acetate is just Sodium acetate and sterile water. I did not adjust PH as recommended in many protocols as a tested PH adjustment prevented the DNA from pelleting well.

Day 1

1. Weigh out ~4 mg of leaf material, put in 2 mL screw top tube, add 40-50 beads.

2. Drop in liquid nitrogen for ~30s, then bead-beat for ~20s

3. For each sample, add 800 µL CTAB Solution #1

per sample	***times _____ samples + 1***
800 µL CTAB	mL CTAB
5 µL Proteinase K	µL Proteinase K
5 µL RNase	µL Rnase A
5 uL BME	uL BME

4. Vortex/shake until no material is still stuck on tubes

5. Place in 50°C bath for at least 30 min.

_____ (time in) _____ (time out)

6. Place in 65°C bath for at least 60 min.

_____ (time in) _____ (time out)

7. Pour solution to fresh 1.5 mL tubes. Place them on ice until chilled.

8. Add 600 µL ice cold chloroform. Invert and release gas from tubes, then rock for 10 min.

9. Centrifuge 10 min at 12000 rpm, then pipette transfer supernatant to a fresh tube.

10. Add 100ul 3M Sodium acetate and invert.

11. Add 800 µL ice cold absolute ethanol and invert a few times.

12. Precipitate at room temp for at least 10 min.

_____ (time in) _____ (time out)

13. Centrifuge 10 min at 12000 rpm to pellet the extraction product.

14. Pour off supernatant carefully, so as not to disturb the pellet.

15. Add 500 µL ice cold 70% ethanol, and rock for 10 min to wash excess salts from pellet.

16. Centrifuge 4 min at 12000 rpm, carefully pour off supernatant, and pipette out remaining EtOH.

17. Dry in Speed-Vac 5 minutes at low heat to dry/ evaporate remaining EtOH.

18. Add 60 µL sterile deionized water, place in 37°C bath or dryer for at least 10 min to resuspend, and flick to mix.

Start pectinase cleaning (Can freeze here and start again another day)

19. Add 740 µL of CTAB Solution #2

per sample	***times _____samples + 1***
740 µL CTAB	mL CTAB
10 mg Pectinase	mg Pectinase

20. Place in 50°C bath overnight

_____ (time in) _____ (time out)

Day 2

21. Place in 65°C bath for at least 30 min.

_____ (time in) _____ (time out)

22. Let tubes cool briefly at room temp. Put tubes on ice until chilled.

23. Add 600 µL ice cold chloroform, invert and release gas, and rock for 10 min.

24. Centrifuge 10 min at 12000 rpm, and transfer supernatant to fresh tube.

25 Add 100ul 3M Sodium acetate and invert.

26. Add 700 µL ice cold absolute ethanol, invert and release gas.

27. Precipitate at room temp for 10 min.

_____(time in) _____(time out)

28. Centrifuge 10 min at 12000 rpm, pour off supernatant, being careful not to disturb pellet

29. Add 500 µL ice cold 70% ethanol, and rock for 10 min.

30. Centrifuge 5 min at 12000 rpm, pour off supernatant, being careful not to disturb pellet, and carefully pipette out remaining ethanol.

31. Dry in Speed-Vac 5 minutes at low heat to dry/ evaporate remaining EtOH.

32. Add 60 µL sterile DI water, place in 37°C bath or dryer for at least 10 min to resuspend, and flick to mix.

33. Run gel at 0.7% agarose concentration.

Appendix 3 - RAD-seq Library Preparation Protocol

DOUBLE INDEX RAD-SEQ LIBRARY PREPARATION

CALBG MOLECULAR LAB

Prepared by Nico Medina - Sep. 2019
Edited by Keir Morse for sequencing a mix of high-quality samples and degraded herbarium samples

Modified from: Etter, P.D., Bassham, S., Hohenlohe, P.A., Johnson, E.A., & Cresko, W.A. (2011). SNP discovery and genotyping for evolutionary genetics using RAD sequencing. Methods in Molecular Biology 772, 157–178. http://doi.org/10.1007/978-1-61779-228-1_9

P5 barcodes from: Tripp et al. 2017. 7(19):7920-7936. doi: 10.1002/ece3.3274

P7 indexes from: IDT_8nt_Unique Dual Indexes_List_V1

Important: do not vortex enzymes and set all reactions in thermocycler. The following protocol assumes you are processing 96 samples for Illumina sequencing combining 24 P5 barcodes and 4 P7 indexes.

Materials:

Step	**Requirements**
1. DNA extractions	300 ng of plant DNA (min concentration 17 ng/µL)
2. Restriction endonuclease digestion	Restriction enzyme (heat inactivated) (PstI 55 ul) 10X NEB Buffer 3.1 (220 ul)
3. P5 adapter ligation	10X NEB Buffer 2 (55ul) T4 DNA Ligase 2,000,000 U/m (27.5 ul) ATP 10 mM (330 ul) P5 barcoded adapters 100 nM
Purification steps	SPRI mag-beads or MinElute PCR Purification Kit (Qiagen)
4. DNA shearing	Covaris sonicator at UCR
5. Size selection	Blue pippin or Ampure XP beads
6. End repair	Quick Blunting Kit NEB - 10X Blunting Buffer (20 ul) - dNTP mix (1mM) (20 ul)

	- Blunt Enzyme Mix (8 ul)
7. 3´-dA overhang addition	NEB Buffer 2 (20 ul) dATP 10 mM (4 ul) Klenow Fragment (3´ to 5´ exo-) 5,000 U/mL (12 ul)
8. P7 adapter ligation	Buffer NEB 2 (20 ul) ATP 10 mM (20 ul) P7 Adapter 10 µM (1 ul x 4 adapters) T4 DNA Ligase 2,000,000 U/mL (2 ul)
9. PCR enrichment	Phusion High-Fidelity PCR Master Mix (312.5+ ul) primer P1 10 µM (6.5+ ul) primer P2 10 µM (6.5+ ul)

1. Normalize concentrations of DNA extractions

For high quality DNA (mostly 1000s of BP):

Dispense 15 uL of $20\ ng/\mu l$ DNA (300 ng) of each sample into strip tubes. Suggestion: sort out your extractions by concentration then add H2O to 15 uL.

For degraded DNA (mostly <1000 BP):
Size select degraded DNA before quantifying concentration to remove DNA smaller than 300bp. Use up to 33 ng/µl DNA (495 ng) for degraded DNA. >33 ng/µl DNA (unknown amount) was used on one sample which yielded the highest # of reads for degraded DNA but only slightly higher than one that was at 33 ng/µl. These yielded no more than average reads compared to high quality DNA in the same lane using 20 $ng/\mu l$.

2. Restriction endonuclease digestion

Prepare the following digestion mix:

	1X	**110X**
10X NEB Buffer 3.1 (may vary according to enzyme)	2 µL	**220 µL**
H20	2.5 µL	**275 µL**
Restriction enzyme (PstI)*	0.5 µL	**55 µL**

*Avoid mixing concentrated buffer with restriction enzyme (add enzyme last)

Add 5 µL of the mix to each DNA sample and incubate for 2.5 hours at 37° C followed by heat inactivation at 80° C for 20 min (temperature may vary according to enzyme used).

If you are concerned about the enzyme itself or inhibitors in your DNA, run a test on a sample with lambda DNA and one of lambda combined with your DNA using equal concentrations of each. If the enzyme works, you should see bands on the gel of different sizes for the lambda digestion (this likely won't be apparent in a digested sample of just your DNA). If your DNA does not inhibit digestion, you should see the same bands in the sample of your DNA combined with lambda.

3. P5 Adapter ligation

Dilute adapters to working concentration (100 nm = 1:99).

Split your samples in 4 batches, each with 24 samples.

For degraded DNA: If some samples aren't to be sonicated as they are already mostly short fragments, keep those all in the same batch and organized so they are easy to split from those that will be sonicated. This protocol assumes those not to be sonicated are only in the 4th batch. If in more batches, adjust the protocol as necessary.

Add 2.5 µL of the P5 adapter to each sample. Make sure to record what barcode you added to each sample. Repeat for each batch.

Prepare the following ligation mix:

	1X	**110X**
10X NEB Buffer 2	0.5 µL	**55 µL**
T4 DNA Ligase 2,000,000 U/m	0.25 µL	**27.5 µL**
ATP 10 mM	3 µL	**330 µL**

Add 3.75 µL of the mix to each deactivated digest. Incubate at room temperature for 30 min, then heat-inactivate ligase for 20 min at 65° C.

Once all samples are barcoded, pool each batch of 24 ligation products, and you should finish with 4 different pools (or more if some won't be sonicated).

For degraded DNA: Keep separate those you don't want sonicated as a 5th pool. Possibly do a bead size selection on this 5th pool if some DNA longer than ~500 that can be added to the 4th pool for sonication, keeping the 300-500 to add back in after sonication.

4. DNA shearing

For the high-quality DNA, shear each pooled sub-library to an average size of 400 bp. If using Covaris sonicator, load up to 5 µg of DNA into a 130 µL tube, one of this per pool should be enough. Following this protocol 130 ul is 1.5 ug of DNA. Covaris tubes are supplied by UCR when using it.

5. Size selection

Following standard protocol, use Blue Pippin instrument or Ampure XP beads to size-select each pooled sub-library to 300-500 bp. Each of the 4 pools should end with 40 ul of product which is the output volume of the Blue Pippin.

*Note that I used the same magnetic bead type for both size selection and cleaning (whatever was in the lab). Not sure if there is an advantage to the two types suggested by the protocol.

For degraded DNA: Add 5th pool back into 4th pool after size selection. Qubit to confirm concentration of each, then use correct ratio based on how much would have been added to sonication tube. Possibly just add a higher concentration of the 5th pool and assume a lot will be lost. It may depend on the size of your degraded DNA fragments.

6. End repair

To 40 µl of the size selected products, add:

5 µl 10X Blunting Buffer
5 µl dNTP mix (1mM)
2.0 µl Blunt Enzyme Mix

Incubate at room temperature for 30 min, heat deactivate for 10 min at 70º C, then clean up with SPRI beads, and elute in 42 µl of H20.

7. 3'-dA overhang addition

1) To the products from the previous step, add:

5.0 µl 10X NEB Buffer 2
1.0 µl dATP (10mM)
3.0 µl Klenow (exo-)

Incubate at 37º C for 30 min. Allow reaction to cool slowly to ambient temperature (15 min), then clean up with SPRI beads, and elute each pool in 42 µl of H20.

8. P7 Adapter ligation

1) To each of the products from the previous step, add:

5.0 µl 10X NEB Buffer 2
1.0 µl P7 Adapter (10 µM)
5 µl ATP (10 mM)
0.5 µl T4 DNA Ligase 2,000,000 U/m

Incubate reaction at room temperature for 30 min, heat inactivate at 65º C for 10 min. Then clean up with SPRI beads and elute each pool in 40 µl of H20. **Do not mix the four pools yet.**

9. PCR Enrichment

Perform a test amplification to determine library quality. In a PCR tube, combine:

10.5 µL H2O
12.5 µL Phusion High-Fidelity Master Mix (expiration date really matters on this, test if close)
0.5 µL primer P1 (10 µM)
0.5 µL primer P2 (10 µM)
1.0 µL RAD library template

(I recommend testing 1, 3, & 5 ul template to find optimal; H_20 + template should = 11.5 ul)

Perform 10 cycles of amplification in thermal cycler: Can go higher if necessary but more cycles = more possible errors

30 sec 98° C
10X: 10 sec 98° C, 30 sec 65° C, 30 sec 72° C
5 min 72° C
hold 4° C

Run 1 µL of PCR product(s) on 1% agarose gel next to 1 µL of RAD library template and 100 bp DNA Ladder. If the amplified product is at least twice as bright as the template, perform a larger volume amplification (2X 50 µl). Previous notes indicate to check concentration of product on Qubit with minimum of 3.6 ng/ul (all pooled) being acceptable if Blue Pippin used as final size selection (possibly ¼ that needed as in 4 larger pools in this protocol). If amplification looks poor, use more library template in a second test PCR reaction. Template should be dim, yet visible on the gel.
* 5 ul worked best for *Malacothamnus* raising template from 0.72 ng/ul to 1.5 ng/ul after PCR.

Clean up the PCR products using Ampure XP beads, aim to remove fragments smaller than 400 bp (0.7:1 beads to PCR mix). Dilute in 42 µL H2O. (maybe 20 ul if your DNA is not very concentrated)

*Your goal is 400-600 bp. Using bead size selection, my gel showed 500-1000 bp with a peak at ~750. For the same sample, the UCR bioanalyzer showed 300-1000 bp with a peak at ~500 and highest concentrations at 400-600 bp. So, don't put too much weight in the accuracy of the gels.

10. Sub-libraries pooling

Quantify DNA of each sub-library using Qubit, normalize concentrations and pool into a single library. Finally, quantify the concentration of the library, concentrate using magnetic beads if necessary* and submit for sequencing**.

*Libraries of 1 ng/µL have resulted in very good data.

**Submit P7 indexes to the sequencing facility. They will demultiplex the data into your four sub-libraries based on this. You will demultiplex each of these based on the P5 adapters.

Appendix 4 - Supplemental Figures

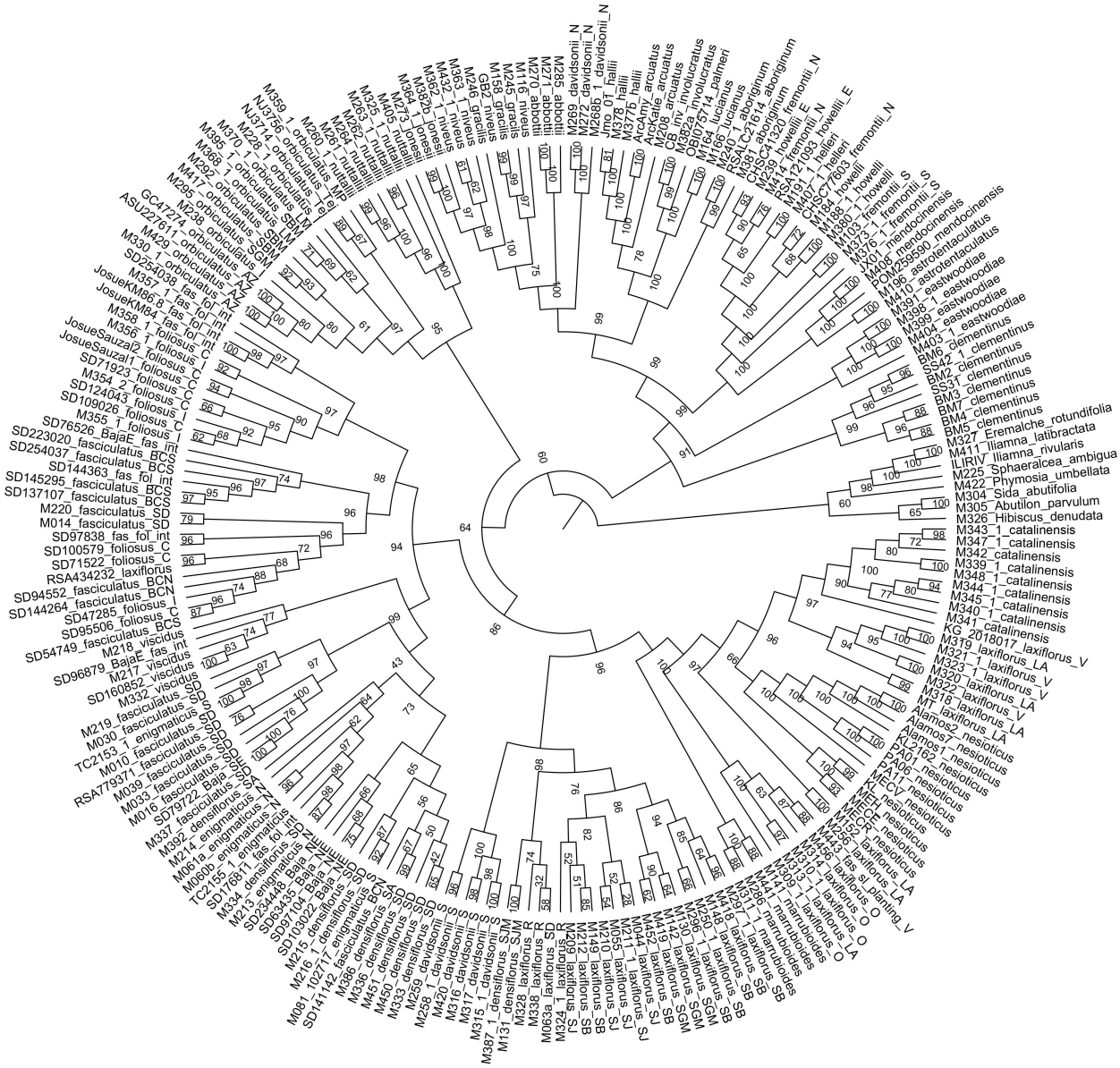

Figure S1. ML tree including only the three untrimmed 150 bp libraries.

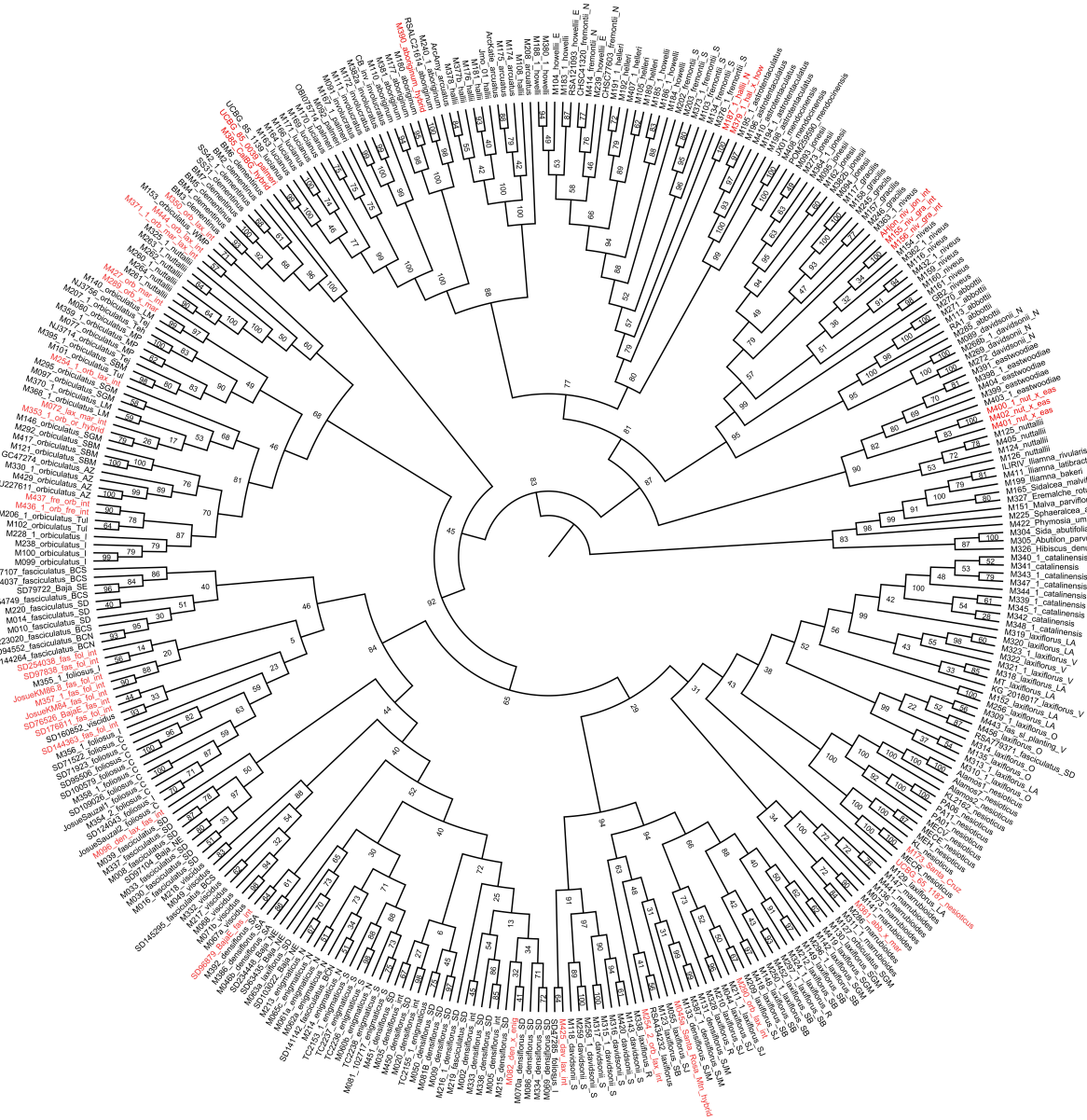

Figure S2. Consensus ML tree of full alignment with hybrids. Likely hybrids are shown in red.

Figure S3. Consensus tree of tetrad analysis of the full dataset without hybrids.